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ADB285843

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USAMRMC ltr, dtd 28 Jul 2003

AD_____

Award Number: DAMD17-96-C-6097

TITLE: Muscle and Liver Carbohydrates: Response to Military Task Performance by Women and Men

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REPORT DATE: October 2001

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2001	Final (23 Sep 96 - 22 Sep 01)

4. TITLE AND SUBTITLE
Muscle and Liver Carbohydrates: Response to Military Task Performance by Women and Men

5. FUNDING NUMBERS
DAMD17-96-C-6097

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, Oct 01). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Throughout this project we have focused on collecting significant data in all specific aims proposed in the original contract. Although hypothesis 3 was redesigned, we have completed the three original hypotheses and the redesigned hypothesis 3. Hypothesis I: We have completed the data analysis and found that, unlike men, women work their lower legs harder during the latter stages of exercise than during the first hour. We have developed a 3-D volume filling protocol that significantly improved the presentation quality of MRI data. One manuscript is in review (EJAP) and another is being written. Hypothesis II: We have confirmed that the left biceps depletes glycogen significantly faster in women (both menstrual phases) than in men ($p=0.0047$, follicular; $p=0.0036$, luteal). We have refuted our earlier preliminary conclusion, that there is a significant menstrual cycle variation in net liver glycogen depletion rates in our female population. A manuscript is currently being written. Hypothesis III: We have observed significantly enhanced muscle glycogen recovery when protein is added to post-exercise carbohydrate supplementation as opposed to isocaloric carbohydrate only supplementation. The manuscript is currently in review (JAP). Hypothesis IV: We have completed this hypothesis and found an increase in glycogen storage over the four days of exercise despite a failure to increase dietary intake. Results were similar in both genders. A manuscript is currently being written.

14. SUBJECT TERMS
women's health, muscle, liver, carbohydrates, military task performance, glycogen, isocaloric

15. NUMBER OF PAGES
117

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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T L B. Pea 6-20-02

PI - Signature Date

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INTRODUCTION

During the course of this project we have endeavored to address four specific aims with the intention of testing the way in which four basic hypotheses relate to gender. **AIM I: To study, in male and female populations, the systemic muscle activity patterns that are associated with a prolonged, non-normalized, repetitive lift and carry task.** We were looking specifically for gender differences during the initial 15min bout of exercise, and over the course of 12 15min bouts (180min) of exercise. We were also seeking to identify muscles that were consistently active in both genders and muscles that were heavily active in one or the other gender. **Hypothesis I:** ***There are differences in muscle activity patterns of men and women when an identical, non-normalized (i.e.-same mass, same duration) repetitive lifting task is performed over a prolonged period.*** We studied twelve subjects (6M, 6F), employing a total body MRI method to examine the effects of lifting (from floor level) and carrying (about 10 feet) a 65lb box at a rate of 3 lifts per minute. The exercise was performed in 12 15min bouts. MRI data were collected before the start of exercise and immediately following each 15min bout. Each 15min bout was separated by no more than 15 minutes. Male and female subjects were age and training matched, and the task was non-normalized (i.e.- both genders were asked to perform the same identical task). We measured the increase in transverse relaxation time (T_2) in 50 different muscles of the upper and lower body. These T_2 increases were noted following each 15min bout over the 180min exercise protocol. We accomplished all technical objectives of this aim. One preliminary manuscript is currently in review (EJAP, see Appendix I), and a second manuscript (the primary manuscript) is currently being written.

AIM II: To study, in male and female populations, the systemic carbohydrate balance associated with a prolonged, non-normalized, repetitive lift and carry task. We were looking specifically for gender differences in carbohydrate metabolic patterns of two muscles and the liver. We were also looking for menstrual cycle variations in the female population. The muscles to be studied were identified from Aim I of this project, and were a muscle that was consistently active (left vastus lateralis) and a muscle that was heavily active in the female population (left biceps brachialis). **Hypothesis II:** ***There are gender differences in systemic carbohydrate balance during the performance of this same prolonged repetitive lifting task and during recovery from the task. These differences are the result of women***

having to work harder to perform the same task. Female menstrual cycle phase may have an effect upon the results. We studied 25 subjects (15M, 10F), employing a NMR spectroscopy (NMRS) method to examine muscle and liver glycogen utilization patterns associated with the previously described 180min lift and carry task. Most of the female population (n=6) was studied on two separate occasions corresponding to the luteal and follicular phases of their menstrual cycle. We failed to accomplish three portions of Aim II as originally stated: 1) Data about glucose transport from ^{31}P NMRS of glucose-6-phosphate, 2) Expired gas analysis during exercise, and 3) Blood chemistry during exercise. ^{31}P NMRS measurements were not made because we did not study the muscle that we originally intended to study. We anticipated that the gasctocnemius would be as consistently active as the quadriceps group; however, this was not the case. So when we opted to study the quadriceps we had to move from a 4.7T limb NMR spectrometer to a 2.1T whole body NMR spectrometer. This meant that we had to choose between measuring either glycogen or glucose-6-phosphate to maintain the desired time resolution. Since the glycogen measurement gave the more basic data, we chose the ^{13}C NMRS measurement of glycogen. The expired gas analysis was not performed during exercise because the when subjects were connected to the expired gas analyzer it interfered with their ability to perform the exercise properly. We assessed work rate from subjects' heart rates as measured by a digital heart rate monitor. Blood samples were collected from the first two subjects that performed this protocol, and it was decided that the IV catheter interfered with their ability to perform the exercise task. We accomplished ^{13}C NMRS glycogen depletion studies in a male population and a female population at two points (luteal and follicular) in their menstrual cycle. Glycogen depletion patterns were studied in two muscles, the left vastus lateralis and the left biceps brachialis, and in the liver (net glycogen concentrations). We were also able to compare data from these muscles performing a systemic exercise protocol with data from a localized (single muscle) exercise protocol performed at three different workloads. However, this data was obtained from a different muscle (gastrocnemius). A manuscript of the data from Aim II is currently being written.

AIM III: To study the effect of administering a carbohydrate supplement before and after a prolonged, non-normalized, repetitive lift and carry task. We were looking specifically for a beneficial effect on carbohydrate utilization in and subsequent recovery of metabolically exercised muscles. We were also looking for gender differences. **Hypothesis III:**

The administration of a carbohydrate supplement immediately before and after the performance of the same prolonged repetitive lifting task may have a glycogen sparing effect, and this effect may be different in men and women. We failed to accomplish any part of the Aim III as originally stated. Because we did not observe a large depletion of glycogen in either muscle studied, nor was there substantial net glycogen depletion in the liver, we felt that a study of the benefits of carbohydrate supplementation with the lift and carry protocol would have been pointless. Aim I, the MRI study, may explain the lack of heavy glycogen depletion in any single muscle, as the work was distributed over so many muscles. In order to accomplish a nutritional supplementation component of this project we redesigned Aim III to employ a cycling exercise protocol that heavily depleted glycogen in a specific muscle group (quadriceps), while also depleting systemic carbohydrates. **Redesigned AIM III: To study the effect of different nutritional supplements, carbohydrate/protein versus carbohydrate only, upon early post-exercise muscle glycogen recovery.** We were looking specifically for any beneficial effect that the addition of protein might have upon muscle glycogen recovery in a group of trained cyclists. **Redesigned Hypothesis III:** ***The addition of protein to a post-exercise nutritional supplement may benefit glycogen recovery during the period immediately following heavy exercise.*** Using ^{13}C NMRS we studied 7 subjects (all trained male cyclists) on three different occasions, comparing the effect of three different nutritional supplements upon glycogen recovery in the vastus lateralis. We accomplished all aspects of the Redesigned Aim III and the manuscript is currently in review (JAP, see Appendix II).

AIM IV: To study the effect of four consecutive days of a prolonged, non-normalized, repetitive lift and carry task on carbohydrate depletion and recovery in exercised muscles. We were looking specifically for any trends in glycogen recovery from day-to-day over the course of the protocol. We were also looking for gender differences in any trends observed. **Hypothesis IV:** ***Four consecutive days' performance of the same prolonged repetitive lifting task causes an overall downward trend in carbohydrate stores. This downward trend is the result of incomplete recovery from each previous day's exercise. The trend may be more pronounced in women than in men.*** We studied 10 subjects (5M, 5F) using NMRS to study glycogen depletion and recovery in the left vastus lateralis and the left biceps brachialis. We failed to accomplish the same three portions of Aim IV as originally stated as described above (Aim II), for the same reasons as stated above. We accomplished ^{13}C NMRS

glycogen depletion studies in a male population and a female population. A manuscript of the data from Aim II is currently being written.

The original intention of this project was to compare the effects of a prolonged, non-normalized, repetitive lift and carry task on age and training matched male and female populations, with particular attention to female menstrual cycle variations. We employed non-invasive methods to study two specific areas; the gender dependence of exercise on muscle activity patterns, and the gender dependence of exercise on muscle and liver carbohydrate metabolism. We have asked questions about the effects of nutritional supplementation upon muscle carbohydrate recovery following exercise, and the effect of four consecutive days of exercise upon day-to-day muscle glycogen recovery. Although we had to redesign one of the original aims, with some minor alterations, the other three aims have been accomplished. This project was extended for an extra year due to difficulties in finding suitable subjects: however, the research has now been successfully completed, and there are two papers currently in review. We have also made numerous presentations at meetings, and we are currently writing three additional manuscripts for submission to a peer-reviewed journal during the next few months.

BODY OF REPORT

During this final period we have focused on completing the research in all areas of the project. We have completed the data analysis and reconstruction for **Aim I**, and we have submitted the first *(European Journal of Applied Physiology)* of two manuscripts to be submitted from this aim. We have completed the research and data analysis for **Aim II**, and are currently writing the manuscript. We have successfully redesigned **Aim III**, completed the research and data analysis, and submitted the manuscript *(Journal of Applied Physiology)*. We have completed the research and data analysis for **Aim IV**, and are currently writing the manuscript. In this section we will condense and report what was done over the course of the entire project.

A: Experimental methods, assumptions, and procedures:

Aim I: Validation of differential muscle recruitment - Exercise induced T_2 changes were compared in two different muscles (gastrocnemius, soleus) that were worked at a constant and standardized workload for a fixed amount of time (1min 45sec). The muscles worked against a constant resistance equal to 25% of the measured maximal voluntary contraction (MVC) for the

gastrocnemius (1). An exercise ergometer that has been employed previously (2) was used to enable dynamic contractions that rotated each muscle through its full range of motion. Each subject performed the same exercise protocol (1min 45sec exercise @ 25% of MVC) on three different occasions with the knee at three different degrees of flexion / extension. Knee orientations were: 1) plantar flexion with the knee fully extended (180°), 2) plantar flexion with the knee flexed at 90°, and 3) plantar flexion with the knee at an intermediate extension (135°). This data has been submitted to the *European Journal of Applied Physiology – MS number SJ 1882/02* and is currently in review. Details of this portion of **Aim I** are in the included manuscript (see Appendix I).

Muscle activity studies in males and females - This study used magnetic resonance imaging (MRI) to compare muscle recruitment patterns in male and female subjects performing a lift and carry exercise protocol. Six males (25±3yrs, 85±3kg, 180±3cm) and six females (27±3yrs, 60±3kg*, 165±3cm*, $p \leq 0.005$ versus males) repeatedly lifted a 30kg (65lb) box from floor level, carried it 3m (10ft), and placed it at a height of 132cm (52in). The exercise protocol was the same for both male and female subjects. Subjects performed 3 lifts/min over a 15min period followed by 15min for data acquisition, during which the subject was allowed to rest. In each study subjects were asked to perform twelve separate 15min bouts of exercise. A repetitive lifting exercise ergometer was constructed from a steel roller assembly of the type routinely used for moving boxes into a warehouse (see Figure 1) (3). The roller was mounted on a frame such that it reached a height of 52in at one end and 5in at the other end. A platform was attached at the lower end to provide a landing, and the entire assembly was mounted on locking rollers for mobility. A box (12in X 12in X 16in) was constructed of high molecular weight plastic (1.5in thickness) with handles mounted at 45° angles (see Figure 1). The box weight (45lbs) was augmented by mounting circular weights (20lbs) with a threaded nylite rod.

T2-weighted transverse MR images (180 slices) were obtained from the whole body before subjects began to exercise and immediately after each bout of exercise. Fifty muscles were assessed for exercise induced T2 increase (4,5). Following each exercise period subjects were positioned at four stations (shoulders, abdomen. pelvis/thighs, and lower legs) during the first 10min immediately following cessation of each block of exercise. Thirty slices were collected about each position (NEX=4, TE=30ms,60ms, total scans = 240 per station). The advantage of a four-atation MRI protocol is that each station was set up and shimmed

individually, thus giving cleaner data.

The body radiofrequency (RF) coil was employed. In all cases, a single shot spin echo echoplanar pulse sequence was used (6). All images were one signal average obtained one at a time at isocenter. Since the table was moving, this resulted in a stack of axial images from head to foot. Slice thickness was 10 mm, slice overlap was 0 mm, and field of view 40 cm by 20 cm. The acquisition matrix was 128 by 128, with the read direction interpolated to 256 in postprocessing. The time between RF pulses was 500 msec, selected using the "TR" parameter on the console; note that since the entire image is obtained in a single shot, and since the slices do not overlap, the actual TR is infinite (except for effects of an imperfect slice selection profile).

Exact subject re-positioning was essential to ensure optimal registration each time the subject was placed in the imager. The subject's feet were placed in a form constructed of foam so that the two lower legs were positioned the same distance apart each time the subject was re-positioned. The subject was given a Lexan rod to hold with his/her hands so that the hands & arms would be held in the same place with each scan, then the subject's upper body & trunk were strapped to the patient bed with a cloth mesh & velcro restraining device, and the subject's knees were bound with a velcro strap. During the initial series of images the patient bed was marked with arrows drawn on tape with a Sharpie, that corresponded to specific anatomical points (ear, elbow, knee) on the subject. During each subsequent re-positioning the subject was placed on the patient bed in alignment with those anatomical markers and strapped in position to prevent movement. The entire re-positioning process required about a minute. The subject was then landmarked, using the electronic alignment hardware on the imager, and placed in the imager. After the completion of the exercise and MRI data acquisition protocol, two stages of data processing were performed to determine muscle activation. These stages were defined as image reconstruction, T_2 calculation, and T_2, and are described below (3):

Image Reconstruction

Due to time constraints during the imaging phase of the exercise protocol, the MRI data was stored in the scanner's computer system in its raw, Fourier domain (K-space), format. This data was processed via a sequence of steps to reconstruct spatial images of the study subject's body. The first step in this sequence is the transfer, via a local computer network, of the raw data from the scanner computer to Unix-based workstations (either Sun or SGI computers), located in the NMR research computer lab. From these computers, the raw data was archived to digital

tape to provide long term storage and to reduce the hard disk storage requirements. The amount of raw data was massive: each raw file contained all of the data for one complete imaging pass through the body (i.e., 180-200 images), each consisting of a matrix of size 256 X n of four byte data values, where n was usually 84 or 114, depending on the TE value used during imaging. Thus a single raw data file could require over 23Mbytes of storage space; given that a single experiment can generate 40 such data files, the data handling and processing was very demanding, both in computer and time resources.

Once the data had been transferred, the next step in image reconstruction was removal of noise from the raw data. The imaging protocol used to collect this data tends to produce occasional "speckle-type" noise (or spikes) in the resulting K-space (frequency domain) data. These spikes must be removed to eliminate striping artifacts in the spatial domain images. The noise removal was achieved using a non-linear filter, similar to a top-hat transform, that detected isolated pixel values much greater or smaller than the values of its neighbors; these spurious values were replaced by an average of the surrounding pixel values. Since K-space data is complex, the real and imaginary component matrices were processed in two separate passes of the filter.

Following noise removal, the K-space data was converted to spatial domain images via a Fourier transform process. Due to the short TE times used in the imaging process, complete K-space data could not be acquired. To reconstruct the partial Fourier images (PFI) without a large loss in resolution, it was preferable to invoke Hermitian symmetry to fill in the missing K-space data prior to Fourier transformation. However, spatially dependent phase shifts, produced by the scanner hardware, can destroy the symmetry of the data. To restore the data symmetry, a 2D version of the Fourier correction algorithm described by McFall, et al., (13) was used. This algorithm estimates the phase correction factors for the partial K-space data using a Fourier transform process applied to the low frequency portion of the collected data. Once the phase factors were computed and applied, restoring the symmetry of the collected data, Hermitian symmetry was used to fill in the missing data. Finally, a Fourier transform was applied to the full K-space data to produce the spatial domain image. The entire reconstruction process, including the denoising filter, was implemented in Matlab (The MathWorks, Inc., Natick,MA). Processing of 150-200 images from a single raw data file required approximately two hours on an SGI IMPACT computer equipped with an R10000 cpu and 128 Mbytes of RAM. The

resulting images consisted of 256 X 128, two byte pixels; all images from a single raw file that were stored in a single file. Images were archived on magneto-optical disks when not needed for further processing.

T_2 Calculation (4)

All T_2 calculations were performed in Matlab (The MathWorks, Inc., Natick, MA) using a software program that was developed "in house" specifically for the processing requirements of this project and designed to enable a more thorough review and evaluation of MR imaging data. The program, which was developed to facilitate selection of regions of interest (ROI's) within muscles for determination of their T_2 values and, therefore, their activation status, was run on a Windows-based PC platform. This software takes as its input an image file containing a series of image slice pairs, where each pair consists of images reconstructed from raw MRI data acquired with a long (60msec) and short (30msec) echo time (TE). T_2 values were computed directly by our ROI sampling software such that average pixel intensities were computed for the selected ROI in each image of an image slice pair, and these average values were then used to compute a single T_2 value for the ROI. The ROI program computed both a raw T_2 value and a corrected T_2 value that accounted for the decay of T_2 value changes over the period of time immediately following the exercise period. The T_2 correction calculations were implemented with the following equations:

$$T_2 = 1/ R_2 \qquad (1)$$

Where: $R_2 = 1/ (TE_1 - TE_2) * \ln (S_1/S_2)$

Where: TE_1 and TE_2 = the two echo times

S_1 and S_2 = the average per pixel signal intensities in the ROI of the image pair slice.

And:

$$T_{2CORR} = T_2 / [1 - \text{correction factor} * (\text{acquisition delay} - 1) * T_2] \qquad (2)$$

Where: correction factor = 0.42msec/min (T_2 decay with time after 1min post-ex)

acquisition delay = [(the time after cessation of exercise) - 1min]

A complete data set (i.e.- the subject completes the exercise protocol) consisted of 13 blocks of data (1 at rest and 1 after each of the 12 15min bouts of exercise, 3 lifts per min = 45 lifts per bout). Each block of data contained four components that corresponded to each of the 4 stations

imaged. Four transients and two echos per slice for 30 slices per station at each of 4 stations yields 960 images per block for 13 blocks, or 12,480 images per study (~1 GB). Data were condensed by averaging the 4 transients together yielding 60 images per station (2 echos per slice) or 120 total slices to be assayed per block of exercise (~200MB of data per study). For each block of data, processing was done slice-by-slice for 50 different muscles (25 each, right and left side of body). Each slice had 3-10 different muscles to assess for T_2, and regions of interest (ROI's) had to be selected so as to avoid vessels, fascia, and MRI artifacts. In order to accumulate a statistically significant number of ROI's for each muscle in a given block of data, ~10 slices per muscle were assessed.

In each muscle, the significance of the change in T_2 value at each time point during the exercise session was tested (paired t-tests) by a comparison of the mean T_2 time obtained for n slices of a given muscle at rest (before beginning the exercise protocol) with the uncorrected mean T_2 time (raw T_2) of n slices of the same muscle obtained at time t after cessation a specific block of exercise. By employing this method of testing for significance, we ran the risk of missing a small increase in T_2 value. However, if the correction factor had been applied before a test of significance was performed, we would have risked introducing an erroneous significant exercise induced T_2 increase. In order to optimize the chances for identifying real T_2 increases, we positioned subjects and began MR scans as quickly as possible following each bout of exercise. In addition, so that any one MRI station would not consistently be imaged following the longest period of time after cessation of exercise, we alternated the order of MRI stations for which we began acquiring images. On average, all images were completed within 10min of cessation of exercise. By acquiring MRI data in this way, we obtained consistent T_2 data over all of the MRI studies.

To generate images that would allow T_2 data to be presented as a three-dimensional color map of the lower leg, two-dimensional MR images of representative male and female subjects were processed using a digital segmentation & reconstruction process. The intention of this type of presentation was to allow readers to get a more global sense of the changes in muscle activation patterns. Selected muscles and bones in transverse serial MRI slices taken from each representative test subject were digitally traced and shaded in cross-sections using Photoshop 5.0 (Adobe Systems, San Jose, CA). The resulting segmented sections were stacked and reconstructed into polygonal models via a marching cubes algorithm, using Synu graphical

software (National Center for Microscopy and Imaging Research at San Diego). The reconstructed muscles and bones were imported into the digital modeling and animation software 3D Studio Max (Kinetix, Milpitas, CA), in which muscles were assigned false colors according to their ΔT_2 values. A linear color scale of indigo through red (hues of 0° — 170° in 3D Studio Max HSB color space) was chosen to represent the measured range of ΔT_2 values of 0 (indigo) to 7.9 (red). Any negative ΔT_2 values were not significantly different from zero and were taken to represent no significant change in T_2. They were therefore assigned a value of zero. These reconstructed images are presented in to portions of this report: Appendix I (Figure 2a), and in Figure 2a-d and Figure 3a-d.

AIM II: Muscle and liver carbohydrate utilization studies – To accomplish this aim we studied 15 males (28±2yrs, 85±3kg, 181±2cm) and 10 females (26±3yrs, 63±3kg*, 167±2cm*, p≤0.0001 versus males) (7). Females were studied during the luteal (3-6da pre-menses) and follicular (3-6da post-menses) phases of their menstrual cycles. Male and female subjects were age and training matched, but not size (weight & height) matched. Male and female subjects, rated according to the Army Physical Fitness Test (Form DA 705), were not significantly different. Composite percentiles were: 84±7 percentile, sit-ups; 92±5 percentile, push-ups; 96±4 percentile, 2-mile run.

The exercise protocol that subjects were asked to perform for Aim II was identical to that of Aim I. One difference from Aim I was that on the day of the study subjects were allowed to eat immediately upon waking (liquid meal) and no further meals were allowed until the exercise session was completed. Baseline NMRS measurements were made in the liver, the left quadriceps muscle group (vastus lateralis), and the left upper arm (biceps brachialis) prior the initial 15min bout of exercise. NMRS data were obtained from one of the three sites following each subsequent 15min bout of exercise, rotating between sites. This was necessitated because of the time required to position the subject and obtain a single NMR spectrum (averaging 13-15min). The exercise protocol was continued until subjects either completed the task or could no longer continue to exercise.

Natural abundance ^{13}C NMR spectra were obtained in 10min blocks using a 2.1 tesla 1m bore spectrometer (Bruker Biospec, Billerica, MA) (8). ^{13}C muscle spectra consisted of 3200 proton decoupled transients using a 90° pulse at 1 coil radius away from a 9cm circular

radiofrequency (RF) coil and a repetition time of 120msec. Proton decoupling was accomplished with an 11cm X 11cm series butterfly RF coil. For liver glycogen measurements a modified ISIS protocol was employed to optimize fat suppression. Subjects were positioned with an image-guided localization MRI protocol (Gradient Echo). Glycogen (GLY) concentrations were determined by comparison with an external standard solution (150mM glycogen + 50mM KCl) that loaded the RF coil the same as the subject (1,13). ^{13}C spectra were processed by methods that have been described in detail in several of our earlier studies (1,8). Briefly, gaussian broadened spectra (30Hz) were baseline-corrected ±300Hz on either side of the 1-^{13}C glycogen resonance of both subject spectra and sample spectra. Areas were then assessed ±100Hz about the resonance and compared.

Revised Aim III: Post-exercise nutritional supplementation studies - This aim was redesigned to severely deplete muscle glycogen in m. quadriceps femoris so that the effect of different nutritional supplements upon muscle glycogen recovery could be compared. The purpose of this aim was to determine the effect of combining protein with carbohydrate in a post-exercise supplement, upon the rate on muscle glycogen storage. Aim III is presented in detail in the submitted manuscript (Appendix II, *Journal of Applied Physiology – MS number JAP-00394-2002*). The methods are described briefly here. Seven trained male cyclists were studied. Subjects were 23±1 years of age (19-26y), 181±1 cm in height (178-183cm), and weighed 74±2 kg (70-82kg). Subjects recorded their dietary intake for 3 days and maintained a cycle training log during the month before the study began (i.e., control period). Maximum oxygen uptakes (VO_{2max}) and maximum heart rates (HR_{max}) were measured using an expired gas analyzer (SensorMedics Vmax 29, Yorba Linda, CA) and Polar © heart monitor (Polar Electro Oy, Finland), at least 72 h prior to the beginning of the study. VO_{2max} and HR_{max} were 61.1±2.1 ml/kg-min (50.6-66.4 ml/kg-min) and 190±4 bpm (178-201 bpm), respectively. Resting heart rates were 44±2 bpm (40-50 bpm). Subjects were then rank ordered according to their aerobic fitness and randomly assigned to the treatment groups.

After the control period, subjects reported to the Yale Medical School, General Clinical Research Center (GCRC) on a Friday afternoon. Subjects were given a normal mixed meal and then asked to fast overnight (12 hrs) while remaining at the GCRC. On the next morning, following baseline NMR and blood measurements, subjects exercised on their own bicycles

equipped with stationary adapters. Each subject performed 2 h of cycling at 65-75 % of his VO_{2max} (9). Oxygen uptake was measured every 30 min and workload adjusted accordingly. During the exercise session, subjects consumed 150ml of water every 15 minutes. After 2 h of cycling subjects performed a series of 1 min sprints at maximum effort. Each sprint was separated by 1 min of rest during which the subject cycled at a self-selected leisurely rate. This sprint phase of the exercise protocol was maintained until the subject's plasma glucose level had dropped below 3.89 mmol/l. This was to insure that liver glycogen stores were depleted to the same degree during each trial thus reducing variability in carbohydrate availability during recovery. There were no significant differences in the total number of sprints completed between the three occasions that each subject performed the exercise protocol. The mean number of sprints completed was 15±2.

In each study subjects were given one of three different nutritional supplements immediately following exercise (within 10 min of cessation) and again 2 h after cessation of exercise. The three different nutritional supplements were: carbohydrate + protein (CHO-PRO), a commercial product (Systems GO International) containing 378 kcal [240 kcal (80g) of carbohydrate + 84 kcal (28g) of protein + 54 kcal (6g) of fat]; isocaloric carbohydrate (HCHO): 378 kcal [324 kcal (108g) of carbohydrate + 54 kcal (6g) of fat]; and isocarbohydrate (LCHO): 294 kcal [240 kcal (80g) of carbohydrate + 54 kcal (6g) of fat]. The nutritional supplements were delivered in liquid form (472 ml) at the two separate time points, for a total of 944 ml (32 fluid ounces) over the 4 h recovery period. Total caloric intake for the CHO-PRO and HCHO supplements was 756 kcal, and for LCHO caloric intake was 588 kcal.

Blood metabolites (insulin, epinephrine, norepinephrine, glucose, lactate, fatty acids) were assessed at rest and during exercise and recovery. Blood samples were drawn from a catheter inserted into an anticubital vein prior to exercise, at 90 min during the exercise, and at 0, 15, 30, 60, 90, 180, 210 and 240 min after each exercise trial. Blood glucose and lactate were measured after every 5 sprints to document exhaustion. Prior to, and every 30 min during the 2-hour exercise trials, heart rate and oxygen consumption were monitored. Muscle glycogen was measured in the thigh muscle (quadriceps) using NMR spectroscopy prior to, immediately (6 min), 15, 30, 45, 60 min, 90 min, 2 hrs, 3 hrs and 4 hrs after each exercise trial. The NMRS procedure used in Aim III was the same as that used in Aim II.

AIM IV: Studies of muscle carbohydrate depletion and recovery on consecutive days of exercise - To accomplish this aim we studied 5 males (25±3yrs, 92±8kg, 185±3cm) and 5 females (21±2yrs. 62±5kg*, 170±3cm*, p≤0.02 versus males). Male and female subjects were age and training matched. but not size (weight & height) matched. Male and female subjects, scored according to the Army Physical Fitness Test (Form DA 705), were not significantly different. Composite percentiles were: 96±3 percentile, sit-ups; 96±2 percentile, push-ups; 100±0 percentile, 2-mile run.

The exercise protocol that subjects were asked to perform for Aim IV was similar those of Aim I and Aim II, with the exception that subjects did not stop exercising after each 15min bout for NMR measurements. Subjects were allowed to exercise in blocks that were comfortable, most subjects choosing to exercise in 30min blocks with 5min breaks. As with Aim II, on the day of the study subjects were allowed to eat immediately upon waking (liquid meal) and no further meals were allowed until the exercise session was completed. Each day, baseline NMRS measurements were made in the left quadriceps muscle group (vastus lateralis) and the left upper arm (biceps brachialis) prior exercise. On each consecutive day of exercise NMRS data were obtained from these two sites following completion of the protocol. On each day the exercise protocol was continued until subjects either completed the task or could no longer continue to exercise. The natural abundance ^{13}C NMR methods employed in Aim IV were the same as those used in Aim II and Aim III.

B. Results:

Aim I: Validation of differential muscle recruitment – The ability of the MRI technique to detect differential muscle activity patterns has been compared with that of the traditional EMG method. We have reported that when a specific muscle activity pattern is altered in a predictable manner, one that has been shown with EMG, the change in pattern is detected with MRI. This is a necessary step in the development of MRI as a technique for studying muscle activity in complex and unknown weight-bearing movement patterns, such as lifting and carrying a weighted box. This study has been submitted for publication *(Eur.J.Appl.Physiol.)*, and is presented in this report as Appendix I.

Muscle activity studies in males and females – This portion of the project was designed to address four basic questions:

1) Are there gender differences in the number of active muscles and the degree of activity?

2) Within each gender, do muscle activity patterns change as exercise continues over 180min?
3) Over the course of exercise what are the muscles that are consistently active in both genders?
4) What muscles exhibit the greatest gender difference in activity?

Results from the MRI studies have been thoroughly analyzed revealing a number of significant gender differences in muscle activity patterns, following the initial 15min exercise bout and over the course of 180min of exercise. We have examined fifty different muscles (25 right and 25 left) in our male and female subject populations. Due to the sheer volume of data, we have not included raw numbers in this report. However, these numbers are available and will be provided upon request. Rather, the data are reported as a color map of individual muscle T_2 increases at four time points (following 15min, 60min, 120min, and 180min of exercise). We believe that this method of presentation offers a more global view of the manner in which muscle activity is initiated and develops over the course of the prolonged exercise protocol. It was the intention of this initial aim to track these patterns according to gender, and to identify the most suitable muscles to be studied in Aims II, III, and IV.

In the male population we have seen that the chest (pectoralis major and pectoralis minor), trunk (rectus abdominus), and the triceps muscles were not significantly active throughout the entire 180min protocol (Figure 2a-d). In the female population however, the right pectoralis major became significantly active ($p \leq 0.05$) 120min into the protocol, while both p. majors and the right r.abdominus were active ($p \leq 0.05$) at 180min (Figure 3c and d). In both genders the lower back muscles were significantly active throughout the 180min protocol (Figure 2a-d and 3a-d).

When the all significantly active muscles examined in this study were grouped and compared according to gender, there was a significant difference between men and women ($p<0.0001$) at all time points for total body muscle activity (Table 1). When muscles were then grouped according to the upper and lower regions of the body, these significant gender differences were maintained (Table 2 and Table 3). Individual muscle pairs (right and left) were grouped and compared according to gender (Table 4 and Table 5). As the exercise protocol progressed gender differences in individual muscle T_2 increases became increasingly significant until at 180min 20 of 28 pairings in the lower body were significant (Table 4). In the upper body the biceps brachialis were consistently more active in the female population ($p<0.03$) (Table 5).

In most muscles studied the degree of muscle activity, measured as individual muscle T_2 increases, exhibited little progressive change (15min – 180min of exercise). In the female population there were significant increases in the activity of some of the lower leg muscles (gastrocnemius and anterior compartment, $p<0.05$) at 180min, while the male population exhibited significant decreases in the activity of some hamstring muscles (biceps femoris and semitendinosus. $p<0.05$) at 180min.

When all data are assessed, we have to conclude that Aim I has been successfully accomplished, and all of the initial questions have been answered in the data analysis. 1) **Are there gender differences in the number of active muscles and the degree of activity?** Yes, following the initial 15min bout of exercise the female population significantly activated 35±2 of the 50 muscles studied, while the male population significantly activated 27±2 muscles ($p=0.031$). The number of significantly active muscles did not appreciably change over the remaining 165min of the protocol, although the variability did increase rendering the gender differences less than significant. There were a number of significant differences in the degree of activity (see above). 2) **Within each gender, do muscle activity patterns change as exercise continues over 180min?** There are some changes but not much. The female population exhibited more reliance on the muscles of the lower leg, while the male population acclimatized to the exercise protocol and relied less on the hamstrings. 3) **Over the course of exercise what are the muscles that are consistently active in both genders?** Yes, the quadriceps were the most consistently active muscle group in the lower body, while the biceps brachii were consistently active in the upper body. 4) **What muscles exhibit the greatest gender difference in activity?** The biceps brachii consistently exhibited the greatest gender difference in muscle activity.

Aim II: Muscle and liver carbohydrate utilization studies – This aim was designed to address six basic questions.

1) How hard must subjects work to accomplish this lift and carry task, and is this reflected in glycogen utilization in the biceps? ..in the quadriceps? ..in the liver?
2) Are there measurable gender differences in glycogen utilization in the biceps? ..in the quadriceps? ..in the liver?
3) Are there measurable menstrual cycle differences in the female population when studied at different points in their menstrual cycle?

4) How does the initial glycogen depletion rate in the biceps and quadriceps (with lift and carry exercise) compare with single muscle data (gastrocnemius) from a localized exercise protocol (plantar flexion) performed at several different workloads?
5) Based on the comparison to the single muscle data, can we say anything about the metabolic workload placed on the biceps and quadriceps by the lift and carry protocol?
6) Can we say anything about the effect of the lift and carry protocol on whole-body carbohydrate metabolism based on the measured net liver glycogen levels during the exercise period?

We completed 35 studies, comparing 15 male subjects with 10 female subjects in the luteal phase of their menstrual cycle and 10 female subjects in the follicular phase. Due to the difficulty of the exercise protocol, some of the female subjects would not consent to return for a second study we were therefore unable to perform paired studies on some of the female subjects. In the female population we performed unpaired comparisons (n=10) on the total subject pool and we performed paired comparisons on two subgroups (n=7 quadriceps, n=5 biceps). We measured glycogen depletion patterns in the left quadriceps muscles (vastus lateralis and vastus intermedius) (Figures 4 - 6) and in the left biceps brachii muscles (biceps and brachalis; Figures 5, 7, 8). We have also measured net glycogen depletion rates in the liver during the prolonged lift and carry protocol (Figure 9).

We measured the glycogen concentration exercise in the left quadriceps (v. lateralis & v. intermedius) every 30min throughout the exercise protocol and found no significant differences in the glycogen depletion patterns (Figure 4). Based on these measurements, we calculated glycogen depletion rates over the entire exercise period. Again, there were no significant differences between genders, nor were there significant differences in the female population at different phases of their menstrual cycle (Figure 5). However, when we compared mid-luteal and mid-follicular glycogen depletion rates during the first 0-60min of exercise, we found a significantly greater mid-follicular depletion rate ($p=0.0227$ paired analysis). We also found a significant reduction in the quadriceps glycogen depletion during the 60-180min period, for both genders and in both phases of the menstrual cycle (Figure 6, $p<0.05$). Because menstrual cycle variations can affect oxidative metabolism, we compared resting glycogen concentrations in the male population and the female population (mid-luteal and mid-follicular). We found a significantly lower resting glycogen concentration in the female mid-luteal population than in the

female mid-follicular and male populations (Figure 7, p=0.0077).

We also measured the glycogen concentration exercise in the left biceps brachii throughout the exercise protocol (Figure 8). We found a significant gender difference in the overall glycogen depletion pattern. During the initial 0-60min of exercise both genders depleted glycogen [Linear regressions (deviation from zero): male p=0.0190, female luteal p=0.0463, female follicular p=0.0329]. However, in the final 60-180min net glycogen depletion in the male population ceased (LR: p=0.1965) whereas it continued in the female population (LR: luteal p=0.0011, follicular p=0.0063). Based on these measurements, we calculated glycogen depletion rates over the entire exercise period and found significant gender differences (Figure 5, luteal p=0.0036, follicular p=0.0047); however, we observed no significant menstrual cycle differences. A comparison resting biceps brachii glycogen concentrations in the male population and the female population (mid-luteal and mid-follicular) revealed no significant differences (Figure 7).

In this laboratory we have a long history of using NMRS to study glycogen depletion with localized exercise. One advantage of studying a single muscle (the gastrocnemius) is that we can isolate essentially all of the work to a single muscle, thereby enabling us to accurately define the workload. Another advantage is that we create an isolated system (<3% of the total body musculature) in an essentially infinite buffer (the rest of the body). This allows us to study local metabolism with a minimum of contribution from the rest of the body. We have amassed a large data-base of glycogen depletion rates at three different workloads (10%, 15%, and 20% of MVC). From this data we have confirmed that a relationship exists between workload and gastrocnemius glycogen depletion rate during the initial period of exercise at these three workloads such that the glycogen depletion rate increases by 0.67mmol/L-hr for every 1% MVC increase above 6% MVC (Figure 9). Unfortunately, this is not possible in a systemic protocol such as the lift and carry exercise employed in this project. However, if we are willing to assume that: 1) other large skeletal muscles respond in a similar manner as the gastrocnemius, and 2) during the early portion of exercise local factors exert the majority of control over local muscle metabolism, we then have a basis for a somewhat tenuous comparison. We believe that under the conditions of the current exercise protocol and with these assumptions, a comparison of the current systemic data from the quadriceps and biceps brachii with our previous localized exercise data from the gastrocnemius is valid. However, we note that numerous other variables may exert

an effect on this comparison, and we are currently gathering data to address some of these other variables. Comparing glycogen depletion rates (0-60min) males worked their quadriceps at ~17% of MVC and worked their biceps at ~20% of MVC. During the luteal phase females worked their quadriceps at ~18% of MVC and worked their biceps at ~25% of MVC. During the follicular phase females worked their quadriceps at ~22% of MVC and worked their biceps at ~32% of MVC.

We measured net liver glycogen concentrations before, during, and after the lift and carry exercise protocol. Liver glycogen depletion rates, calculated based time points that were collected >5hr post-meal, were not significantly different between genders, nor were they different in the female population at different phases of their menstrual cycle (Figure 10). In this protocol net liver glycogen depletion rates were also not significantly different from typical fasting net rates at rest (10).

We conclude that Aim II has been successfully accomplished, and all of the initial questions have been answered in the data analysis. **1) How hard must subjects work to accomplish this lift and carry task, and is this reflected in glycogen utilization in the biceps? ..in the quadriceps? ..in the liver?** Male subjects worked at a significantly lower heart rate (60±2%, $p<0.0035$) with 80% completing the 180min exercise protocol (2.8±0.5 hours completed, $p<0.0045$ versus females in both menstrual phases). Females worked at 74±4% (mid-luteal) and 73±3% (mid-follicular) of their maximum heart rate. During the luteal phase 33% completed the 180min exercise protocol (1.9±0.3 hours completed), and in the follicular phase 13% completed the 180min protocol (1.5±0.3 hours completed). Metabolically, individual muscles were not heavily worked, with initial workloads ranging between 17 and 32% of MVC. This may have resulted from the manner in which the total workload was distributed amongst up to 35 different muscles. The liver was not significantly challenged by this exercise protocol. **2) Are there measurable gender differences in glycogen utilization in the biceps? ..in the quadriceps? ..in the liver?** Yes, in the female population the biceps had to work harder metabolically to accomplish the task, whereas there were no significant metabolic gender differences in the quadriceps or the liver. **3) Are there measurable menstrual cycle differences in the female population when studied at different points in their menstrual cycle?** Yes, in the quadriceps mid-follicular glycogen depletion rates were significantly greater during the initial 0-60min of exercise. We did not observe menstrual cycle differences in biceps glycogen

depletion rates or in net liver depletion rates. **4) How does the initial glycogen depletion rate in the biceps and quadriceps (with lift and carry exercise) compare with single muscle data (gastrocnemius) from a localized exercise protocol (plantar flexion) performed at several different workloads?** We made this comparison based on a couple of assumptions (see above) and found that the biceps were working at a greater % of MVC (20-32%) than the quadriceps (17-25%). **5) Based on the comparison to the single muscle data, can we say anything about the metabolic workload placed on the biceps and quadriceps by the lift and carry protocol?** Yes, in both genders neither muscle was heavily challenged by this exercise protocol. **6) Can we say anything about the effect of the lift and carry protocol on whole-body carbohydrate metabolism based on the measured net liver glycogen levels during the exercise period?** Yes, the measured net liver glycogen depletion rates indicate that whole body carbohydrate reserves are not heavily challenged by this exercise protocol.

Revised Aim III: Post-exercise nutritional supplementation studies – The effect of three different nutritional supplements to aid muscle glycogen recovery during the initial recovery period (0-240min) following heavy systemic glycogen depletion. We found that, when compared with an isocaloric carbohydrate only supplement, the addition of protein to a carbohydrate supplement enables heavily depleted muscles to recover more glycogen during early recovery. These results, which are currently in review in the *Journal of Applied Physiology*, are presented in detail in Appendix II of this report. This Aim, although not the original one, has been successfully accomplished.

AIM IV: Studies of muscle carbohydrate depletion and recovery on consecutive days of exercise – This aim was designed to address four basic questions.

1) What is the effect of four consecutive days of prolonged (180min) lift and carry exercise on glycogen depletion / recovery patterns in exercised muscles (i.e. – is there an overall downward trend from incomplete day-to-day glycogen recovery)?
2) Do gender differences exist?
3) What is the ability of males and females to complete the four day task?
4) How hard do they have to work?

We completed 10 studies (5M, 5F) following glycogen depletion and recovery in the quadriceps (v. lateralis and v. intermedius) and the biceps brachii. Over the course of the four day protocol the male subjects worked at an average of 55±2% of their maximum heart rate,

while the female subjects worked at a higher rate (67±4% of their maximum heart rate, p=0.0196). The male population completed 89±5% of the total 720min (4da) of the exercise protocol, with one subject completing the entire 720min. The female population completed significantly less of the total 720min (53±14%, p=0.0322), with no subjects completing the entire 720min. There were no significant gender differences in the pattern of glycogen depletion and recovery in either the quadriceps or the biceps brachii (Figure 11). The muscle glycogen data were therefore pooled for the two muscles. When the day-to-day depletion and recovery patterns were assessed for the quadriceps the pre-exercise glycogen concentration on day 4 (116±10mmol/L, p=0.0463) was significantly greater that the pre-exercise glycogen concentration on day 1 (92±9mmol/L), indicating significant glycogen super-compensation in the quadriceps (Figure 12). Super-compensation in the biceps brachii occurred by day 3 (62±4mmol/L, day 1; 75±5mmol/L, day 3, p=0.0274), and was greater on day 4 (82±5mmol/L, p=0.0045) (Figure 12). Subjects were asked to record their dietary intake for 4 weeks prior to the study, and during the 4-day protocol. There were no significant differences in dietary intake before the study as compared to during the study. Super-compensation occurred despite the absence of an increase in dietary intake.

Aim IV has been successfully accomplished, and all of the initial questions have been answered. **1) What is the effect of four consecutive days of prolonged (180min) lift and carry exercise on glycogen depletion / recovery patterns in exercised muscles (i.e. – is there an overall downward trend from incomplete day-to-day glycogen recovery)?** Despite no increases in dietary intake, muscle glycogen is progressively super-compensated in the day-to-day recovery from four consecutive days lift and carry exercise. **2) Do gender differences exist?** No. While gender differences likely exist in the glycogen depletion rates, as shown in Aim II, we found no gender differences in day-to-day glycogen recovery. **3) What is the ability of males and females to complete the four-day task?** The male population completed a significantly larger percentage of the total (720min over 4 days) exercise protocol. **4) How hard do they have to work?** The females worked at a greater percent of their maximum heart rate.

C. Discussion:

During this project we have collected enough data to allow us to draw several basic conclusions about gender differences during our prolonged exercise protocol. We have studied the four basic hypotheses that were originally proposed and contracted, including a revised

version of hypothesis III. Our results and conclusions are discussed below:

Hypothesis I: ***There are differences in muscle activity patterns of men and women when an identical, non-normalized (i.e.-same mass, same duration) repetitive lifting task is performed over a prolonged period.*** The prolonged repetitive lifting task can be accomplished by both genders; however, a smaller percentage of women (33%) than men (100%) are able to complete all 180min of exercise. The T2 increases in women are universally greater in women than in men, indicating that women must work harder to accomplish the same task (Table 1-5, Figure 2a-d & 3a-d). Women must also use a greater number of muscles to accomplish the task. As the exercise protocol proceeds into the latter stages (120-180min) men do not have to work any harder than they did at the start of exercise (0-15min); however, in the female population there is a trend toward increasingly harder work (Figure 2a-d & 3a-d). We conclude that both men and women distribute a prolonged repetitive lifting task over a large number of muscles (more in women than in men), so that the total workload is shared and no individual muscle is heavily recruited. Furthermore, we conclude that women work all of the muscle groups that were studied harder than do men, and as exercise extends over a prolonged period women may need to work even harder. However, both genders are capable of accomplishing the task, and size appears to be the most important controlling factor.

Hypothesis II: ***There are gender differences in systemic carbohydrate balance during the performance of this same prolonged repetitive lifting task and during recovery from the task. These differences are the result of women having to work harder to perform the same task. Female menstrual cycle phase may have an effect upon the results.*** We have measured muscle glycogen depletion in the liver, thighs and upper arms of men and women in the luteal & follicular phases of their menstrual cycles. We found no significant differences between the three groups in glycogen depletion rates in the thighs (m.vastus lateralis & m.vastus intermedius); however, resting glycogen concentrations were significantly lower in the mid-luteal female population (Figure 7). It makes sense that muscles might store more carbohydrate during the follicular phase of the menstrual cycle, given that they do not burn fat as efficiently as in the luteal phase. The observation that glycogen depletion rates are moderate and are not gender or menstrual cycle dependent is suggestive of muscles that are not having to work hard to perform the specified work. This makes sense, given that the total workload is distributed amongst so many muscles (see results of Hypothesis I). Based upon single muscle glycogen

depletion data collected in our laboratory over a decade, we calculate that the thigh muscles of both genders were working at less than 25% (17% male, 18% female luteal, 22% female follicular) of their maximum capacity to accomplish our lifting & carrying task (Figure 9). The apparently greater workload during the follicular phase was not significant; however, it is in agreement with a greater reliance on carbohydrate stores in the follicular phase. When we compared glycogen depletion rates during the first hour of exercise with rates during the subsequent two hours we found that in all three populations glycogen depletion slowed significantly (Figure 6).

Data on the biceps suggest that, while the men do not work their left biceps muscles heavily (less than their quadriceps), women deplete glycogen in their biceps muscles at a significantly higher rate than their quadriceps muscles (luteal $p=0.0284$, follicular $p=0.0213$). Furthermore, women in both phases of their menstrual cycle deplete biceps glycogen at a significantly greater rate than men (Figure 5). A comparison with our single muscle glycogen depletion data suggests that women are working at 25% (luteal) and 32% (follicular) of their maximum capacity, while men are working at only 20%. These biceps glycogen depletion rates support the prediction of our MRI data (Aim I) that demonstrates greater recruitment of the left biceps in the female population. As with the quadriceps, male glycogen depletion rates were the same or slower during the last 120min of exercise than during the first 60min (Figure 8). However, during both phases of their menstrual cycle female glycogen depletion rates were maintained in the final 120min of exercise (Figure 8). Taken as a whole, muscle glycogen measurements in both of the muscles that we studied indicate that glycogen is not severely depleted in either gender with our prolonged lift and carry protocol. Therefore, fatigue during this type of exercise is not likely to result from muscle glycogen depletion in either gender.

The net liver glycogen measurements indicate that there are no significant differences in net liver glycogen depletion in the three groups studied (Figure 10). As we discussed in previous yearly reports, the liver measurements are complicated by the liver's ability to turnover its glycogen stores so that blood glucose concentrations can be maintained. Muscles do not have the enzyme necessary to cleave the terminal phosphate group from glucose-6-phosphate (glucose-6-phosphatase) and allow the release of glucose from muscle cells. The liver also has the ability to rapidly synthesize glycogen from three-carbon compounds generated by muscle metabolism and taken up by the liver (gluconeogenesis). Therefore, our measurements cannot

track turnover and only indicate the net liver glycogen concentration at the time of measurement. However, by obtaining these net liver glycogen measurements we can get some sense of the systemic carbohydrate balance that is moderated by the liver. Our data indicate that the prolonged lift and carry exercise protocol does not significantly challenge the liver's ability to maintain readily available carbohydrate stores. Furthermore, the muscle and liver data obtained from both men and woman in this study indicate that with this specific lift and carry exercise protocol fatigue does not result from a depletion of carbohydrate reserves.

Redesigned Hypothesis III: ***The addition of protein to a post-exercise nutritional supplement may benefit glycogen recovery during the period immediately following heavy exercise.*** We have measured muscle glycogen recovery in the thighs (m.vastus lateralis and m.vastus intermedius) of trained cyclists following an exhaustive bout of cycling exercise that depleted greater than 100mmoles of glycogen. A comparison under different nutritional conditions revealed that when a mixture of carbohydrate and protein is administered [~75% carbohydrate (80g) + ~25% protein (28g)] glycogen recovery is enhanced during the first 40min and after 240min following exercise as compared with an iso-caloric amount of carbohydrate alone (108g) (see Appendix II). We believe that the reason for the protein enhancement of muscle glycogen recovery may be an increased insulin response to the addition of protein into the nutritional supplement; however, we were unable to detect significant increases in plasma insulin samples. We do have the results of glucose analysis that reveal significantly lower blood glucose levels during the first hour of recovery by subjects given the carbohydrate + protein supplement. The glucose results suggest that there may have been an enhanced insulin response in this group that increased the glucose clearance rate. It is possible that there was an increase in bound insulin that was not detectable in the plasma samples. This data indicate that nutritional supplements that contain a protein component provide an enhanced ergogenic effect during early recovery. The results from this hypothesis are discussed in more detail in the discussion section of Appendix II.

Hypothesis IV: ***Four consecutive days' performance of the same prolonged repetitive lifting task causes an overall downward trend in carbohydrate stores. This downward trend is the result of incomplete recovery from each previous day's exercise. The trend may be more pronounced in women than in men.*** We have now completed our study to examine the effect of performing our prolonged lift and carry exercise protocol on four consecutive days. Our data indicate a progressive super-compensation that becomes significant as the protocol continues

over four days (Figure 11). This super-compensation occurs in the absence of a high carbohydrate diet, and suggests that the body can completely recover from the protocol overnight and prepare for the next day's demands. We have compared genders and found similar patterns in men and women (Figure 12).

D. Relationship to the Statement of Work (SOW) outlined in the proposal:

While we were unable to accomplish the total number of studies that were originally proposed, we were able to streamline the experiments in such a way that the three of the four original hypotheses were accomplished. The original hypothesis that was not accomplished was not deleted from the project; rather, it was redesigned to reflect a more appropriate goal base upon the results that we were getting. Therefore, we report that all of the hypotheses were accomplished. Based upon power analysis of the data that we collected, we believe that the conclusions that were drawn from this project would not have benefited from completing the number of studies that were originally proposed. Therefore, we believe that our results are in line with the SOW.

E. Negative results:

Aside from the difficulties of subject recruitment for this rigorous protocol, we have experienced no negative results in accomplishing the experiments. During the third year of the project we had one subject who complained of a persistence of soreness as a result of the study, and we reported the incident to the human investigation committee. When the subject complained we advised him that if he felt that his participation in the study had caused a problem he should report the problem. We further advised him that, as indicated in his signed subject consent form, he was entitled to any treatment deemed necessary as the result of problems associated with his participation in the study. He informed us at that time that he did not feel that his persistent soreness would be a problem.

F. Problems in accomplishing tasks:

The problems that were reported in our earlier annual reports have persisted throughout the project. The problem of subject recruitment was particularly bothersome; however, the increase in subject pay helped us to complete the project. In order to complete the project we were required to continue our studies beyond the term of the contract. However, this persistence

has yielded a successful completion to this project.

KEY RESEARCH ACCOMPLISHMENTS

Year 1:

- Developed an echo-planar MRI protocol
- Designed and constructed a lift & carry exercise apparatus

Year 2:

- Demonstrated that MRI is capable of detecting gender differences in the lift & carry task
- Demonstrated that MRI can point to the muscles best studied with MRS
- Determined that liver glycogen is not significantly depleted by the lift & carry protocol in either gender
- Determined that quadriceps muscle glycogen is nominally depleted by the lift & carry protocol in both genders

Year 3:

- Demonstrated with MRI that women performing the lift & carry protocol recruit their upper body muscles, particularly arms, to a greater extent than do men
- Demonstrated with MRI that as the prolonged lift & carry protocol continues the whole body muscle recruitment pattern remains the same as following the initial exercise period
- Demonstrated with MRI that the quadriceps muscles are consistently recruited by the lift & carry protocol in both genders
- Demonstrated with MRI that the biceps muscles are recruited to a significantly greater extent by the lift & carry protocol in women
- Determined that the biceps would provide the greatest opportunity to demonstrate metabolic gender differences during the lift & carry protocol using MRS

- Determined that with the current variability gender differences in biceps glycogen depletion rates will be demonstrated in this project

Year 4:

- Demonstrated with MRI that in women, as prolonged exercise continues into the third hour, the change in T_2 increases in the lower legs indicating that they are working harder
- Developed a volume filling process the allows multi-slice 2-D transverse MR images to be rendered in 3-D, thereby allowing T_2 data to be dumped into reconstructed male and female images of subjects from the study
- Demonstrated with MRS that during the prolonged lift & carry task women deplete glycogen from their biceps muscles at a significantly greater rate than do men, thereby confirming the prediction of the MRI study
- Reversed the previous demonstration of menstrual cycle variations (luteal phase) in liver glycogen depletion rates
- Demonstrated with MRS and blood analysis that adding protein to post-exercise nutritional supplements may provide a benefit

Extension period (Year 5):

- Demonstrated a significant reduction in resting glycogen in mid-luteal females.
- Demonstrated, in both genders, a bi-phasic glycogen depletion pattern in the quadriceps with a reduced depletion rate during the 60-180min period of prolonged lift & carry exercise.
- Demonstrated this same bi-phasic glycogen depletion pattern exists in the biceps brachii of men, but not in the biceps brachii of women during prolonged lift & carry exercise.
- Calculated a predicted workload for the quadriceps and biceps brachii during prolonged lift & carry exercise by comparing current data with single muscle exercise data collected in our laboratory.

- Demonstrated an ergogenic effect of a carbohydrate + protein nutritional supplement compared with an iso-caloric carbohydrate only supplement during the initial period of recovery following heavy exercise (systemic carbohydrate depletion).
- Demonstrated a progressive super-compensation of muscle glycogen that becomes significant in the latter stages of four consecutive days of prolonged lift & carry exercise.

REPORTABLE OUTCOMES

1999: (July) Women's Health Research Workshop Series, National Center of Excellence in Women's Health at Yale. Title: "Equality in the Military: Will All Jobs be Open to Women?" [PRESENTATION]

2000: (September 20-23, Portland, ME) American Physiological Society Intersociety Meeting, The Integrative Biology of Exercise, [ABSTRACT entitled: "MRI of Gender Differences in Exercise" APS Quarterly 43(4), p.362.

2000: (September) National Geographic, Section entitled "The Inner Workings of Fitness", pp. 14-15, in an article entitled "What it Takes to Build the Unbeatable Body: Pushing the Limits" by Rick Gore. This section uses the 3-D volume filling technique that will be used to present our MRI data in publication.

2001: (October 4-6, Marseille, France) International Workshop on Non-Invasive Investigation of Muscle Function, Plenary Lecture Title: "Carbon-13 NMR Spectroscopy of Muscle Physiology"

2002: (April) Departmental Seminar Presentation, Yale Department of Diagnostic Radiology. Title: "Gender Differences with Prolonged Exercise: Final Report on the U.S. Army Study"

2002: (May 28-June1, St. Louis, MO) American College of Sports Medicine 49th Annual Meeting, Mini-Symposium entitled: "Muscle Activity Localization Using MRI", Talk Title: "MRI- and EMG-Based Studies of Muscle Activation During Exercise", ACSM Program Booklet, p.139.

2002: (April) **Price,T.B.**, G. Kamen, B.Applegate, B.Damon, C. Knight, J.C.Gore. K.Eward, and J.F.Signorile. Validation of MRI to study muscle recruitment by dynamic plantar flexion: Comparison with EMG. Eur. J. Appl. Physiol. (in review) 2002.

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CONCLUSIONS

We conclude that, while women must work harder to accomplish the non-normalized prolonged lift & carry task, the result is much more a function of size than of gender. This is particularly true in the upper arms, where biceps T_2 increases are significantly greater, and glycogen depletion rates during the lift and carry protocol are significantly faster in women than in men. This finding represents the first time that MRS has been used to confirm MRI as a means of predicting muscle glycogen metabolism. Furthermore, we conclude that there is a beneficial effect of adding protein to post-exercise nutritional supplementation. We also conclude that in both genders, and during four consecutive days of prolonged lift & carry exercise, there is a progressive super-compensation in day-to-day glycogen recovery despite a lack of increase in dietary intake. This super-compensation becomes significant during the final two days. Finally, we conclude that size matters, and it is only because women are typically smaller than men that gender may matter.

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UNPUBLISHED DATA (all results, discussion, appendices, tables, and figures) related to the second portion of Aim I, Aim II, and Aim IV.

Appendices:

Tables:

Figures:

lowest significant T_2 increase, on the right indicates the highest T_2 increase, and in the middle indicates the mean T_2 increase. Muscles that are colored gray represent muscles that were assessed and found not to be significantly active.

a. Following the initial 15min of exercise. **(pg.105)**
b. Following 60min of exercise. **(pg.106)**
c. Following 120min of exercise. **(pg.107)**
d. At the end of the exercise protocol (180min of exercise). **(pg.108)**

Figure 4: Time course of left quadriceps glycogen concentrations [mmol/L] in the male (■), female mid-luteal (Δ), and female mid-follicular (○) populations over the 180min lift and carry exercise protocol. Mean±SE. **(pg.109)**

Figure 5: Muscle glycogen depletion rates [mmol/L-hr] in the male and female populations for the left quadriceps and left biceps brachialis. Glycogen depletion rates in the biceps brachialis were significantly greater for the females in both menstrual phases. Mean±SE. **(pg.110)**

Figure 6: Left quadriceps glycogen depletion rates [mmol/L-hr] during the initial 0-60min of exercise and during the final 60-180min of exercise. Glycogen depletion rates in both genders were significantly slower during the final 60-180min of exercise. Mean±SE. **(pg.111)**

Figure 7: Resting glycogen concentrations [mmol/L] in the left quadriceps and the left biceps brachialis of both genders. Resting glycogen was significantly lower in the mid-luteal females. Mean±SE. **(pg.112)**

Figure 8: Time course of left biceps brachialis glycogen concentrations [mmol/L] in the male (■), female mid-luteal (Δ), and female mid-follicular (○) populations over the 180min lift and carry exercise protocol. Mean±SE. **(pg.113)**

Figure 9: Plot of the relationship between gastrocnemius glycogen depletion rates [mmol/L-hr] during localized exercise and workload at 10, 15, and 20% of MVC, with linear regression. From 6% through 20% of MVC the depletion rate increases by 0.67mmol/L-hr per %MVC. **(pg.114)**

Thomas B. Price, Gary Kamen, Bruce M. Damon, Christopher A. Knight, Brooks Applegate, John C. Gore, Ken Eward, and Joseph F. Signorile

Comparison of MRI and EMG to study muscle activation by dynamic plantar flexion

Running head: Muscle activation by dynamic plantar flexion

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ABSTRACT

This study compared magnetic resonance imaging (MRI) and surface electromyography (EMG) to evaluate the effect of knee angle upon activation of the triceps surae muscles [medial & lateral gastrocnemius (MG, LG) and the soleus (SOL)] by plantar flexion. Two weight & height matched groups participated, twelve (6M, 6F) in the MRI group, twelve (8M, 4F) in an EMG group that performed an identical protocol. Subjects plantar flexed dynamically for 2min at 25% of their 1-repetition maximum voluntary contraction (1-RM). Exercise sessions were performed with the knee extended (0° flexion), flexed (90°), and partially flexed (45°). In the MRI group spin-echo images were acquired before and immediately following each exercise session. T_2 times, calculated at rest and after exercise by fitting the echoes to a monoexponential decay pattern with a least-squares algorithm, were compared with EMG data. In the EMG group a bipolar electrode was used to collect EMG samples were from the MG, LG, SOL, and anterior tibialis (TA) during exercise at each knee angle. MRI also examined the peroneus (PER). At 0° knee flexion MRI demonstrated a significant post-exercise T_2 increase in the MG ($p \leq 0.001$), LG ($p \leq 0.001$), and PER ($p \leq 0.01$), with no T_2 change in the SOL or TA. At 90° knee flexion there was a significant T_2 increase in the SOL ($p \leq 0.001$) with no significant T_2 change in the MG, LG, PER, or TA. At 45° T_2 increased significantly in the SOL ($p \leq 0.001$) and LG ($p \leq 0.05$), but not the MG, PER, or TA. The EMG protocol produced similar results with the exception that there was significant activation of the TA during the relaxation cycle of the 90° protocol. We conclude that: 1) MRI and EMG produce similar results from different physiological sources, and are therefore complementary tools for evaluating muscle activity; and 2) Muscle activation during plantar flexion is a shared phenomenon with the participation of prime movers being affected by the degree of flexion of the knee.

Keywords: MRI, EMG, motor unit activation, exercise, electromyography

INTRODUCTION

The medial and lateral gastrocnemius (MG & LG) and soleus (SOL) muscles make up a synergistic group known as the triceps surae that actively contributes to human locomotion (Weineck 1990). Comparisons among the MG, LG, and SOL are interesting because of their common insertion on the posterior surface of the calcaneus via the Achilles tendon (Weineck 1990). While the triceps surae muscles all plantar flex the foot, they are different anatomically, biochemically, and functionally (Bilodeau et al. 1994; Capady and Stein 1987; Johnson et al. 1973; O'Rahilly 1983). To maintain a standing posture, the monoarticular SOL is always active to counter the effect of gravity (Galley and Forster 1987). The postural role of the SOL is supported by its mainly Type I fiber composition that is capable of aerobically maintaining prolonged isometric tension (Galley and Forster 1987; Johnson et al. 1973). The MG and LG have a higher percentage of Type II fibers that can generate the high rates of force development required for more dynamic activities (Galley and Forster 1987; Johnson et al. 1973). Because the MG and LG originate on the femur, the gastrocnemius can produce more leverage than the SOL (Sale 1982). Because they are bi-articular, they are more strongly affected by knee joint angle than the SOL, becoming longer to generate more leverage with the knee extended (Sale 1982) and shortening as the knee is flexed. Recruitment of the triceps surae is increased as activity levels increase from standing to walking to running and jumping (Capady and Stein 1987; Galley and Forster 1987; Moritani and Muro 1990).

Historically, electromyography (EMG) has been considered the "gold standard" for studying muscle activation. EMG was recently used to study the functions of the MG and LG, and the SOL muscles during fast running, demonstrating that, when compared to the soleus, EMG activity increased in the gastrocnemius much sooner following ground contact (Jacobs et al. 1993). During the initiation of ground contact the knee shows greater extension than at any other point of the running stride, and it is at full knee extension that the triceps surae are capable of generating the most force (Cresswell et al. 1995). Numerous studies employing EMG have demonstrated selective activation during various tasks involving plantar flexion (Bilodeau et al. 1994; Capady and Stein 1987; Jacobs et al. 1993; Moritani and Muro 1990; Nardone and Schieppati 1988; Nardone 1989; Sale 1982). EMG was recently used to demonstrate that muscle activation patterns in the triceps surae are dependent upon knee angles (Tamaki et al. 1997); however, this has not been demonstrated using MRI.

T_2-weighted MR images can be used to measure muscle activation within groups of muscles, which appears as a differential increase in MR properties that is workload dependent (Fisher et al. 1991; Price et al. 1995; Price and Gore 1998; Sloniger et al. 1998). These exercise induced signal changes result primarily from increases in the spin-spin (T_2) relaxation time of tissue water (Fisher et al. 1991; Fleckenstein et al. 1988; Price and Gore 1998). Therefore, while EMG measures a change in electrical potential occurring along a muscle during muscular contraction, MRI depends on the resulting shift in water brought about by metabolic changes during the contraction. MRI has been used to demonstrate activation of the gastrocnemius by plantar flexion (Fisher et al. 1991; Fleckenstein et al. 1988; Fleckenstein et al. 1989; Price et al. 1995; Price and Gore 1998;

Vandenborne et al. 2000); however, activation of the soleus has not been seen with MRI. The purpose of this study was twofold: 1) to demonstrate a measurable plantar flexion induced signal increase in the soleus using MRI, and 2) to compare MRI and EMG as tools for studying patterns of muscle activation. We employed MRI and EMG techniques to assess lower leg muscle activation during two minutes of repeated plantar flexion's performed at 25% of 1-repetition maximum voluntary contraction (1-RM) and examined the effect of different degrees of knee flexion upon muscle activation patterns.

METHODS

SUBJECTS:

MRI: Twelve healthy subjects (6 male, 6 female) participated in the MRI portion of this study. The MRI subjects were 30±3 years of age, 72±5kg weight, and 172±3cm in height. Subjects were screened according to the Yale-New Haven Hospital standard clinical criterion for MR imaging. This criterion requires that, when accepted into the study, each MRI subject provided informed written consent in accordance with a protocol approved by the Yale University Human Investigations Committee.

EMG: Twelve healthy subjects (8 male, 4 female) participated in the EMG portion of the study. Subjects were 23±3 years of age, 77±5kg weight, and 173±2cm in height. Each subject provided informed consent in accordance with Human Studies Committee of the University of Massachusetts at Amherst.

EXERCISE PROTOCOLS:

MRI and EMG: Using MRI exercise induced T_2 changes were assessed in the lower leg MG & LG, SOL, anterior tibialis (TA), and peroneus (PER). These muscles were dynamically plantar flexed at a constant and standardized workload of 25% of 1-RM [1-RM = the maximum one-repetition plantar flexion load] for a fixed amount of time (2min) (60 contractions). Subject 1-RM values were determined at full knee extension (0°) and found to be 1284±69N (666-1646N) (Price et al. 1991; Price et al. 1995). Subjects exercised in a random testing order with the knee flexed at either 90°, 45°, or 0° (0° = full extension, Figure 2a) at an angular contraction velocity around the ankle of ~45°/s. Two-minute exercise protocols were performed in the same leg in exercise sessions that were separated by at least one hour. Electrical activation, measured with EMG, ceases immediately at the end of each contraction, while the water movements that are measured with MRI require more time to return to the basal state (Price and Gore 1998). During exercise, muscles contracted so as to rotate the ankle through a full range of motion (Williams and Stutzman 1959) against a constant resistance equal to 25% of 1-RM for the plantar flexion. The average rate of contractions was 30 per minute (~60 total contractions). A single mid-calf MR image (single-slice) was obtained immediately before and after each exercise protocol.

The exercise ergometer was designed to enable dynamic contractions that rotated muscles through a full range of motion against a constant resistance, and has been previously described (Price et al. 1995). Briefly, the ergometer was constructed from non-magnetic materials and mounted to a standard MRI patient table, to permit exercise

within the MR imager. To provide constant resistance, a sealed dual-chambered pneumatic cylinder/piston assembly was articulated with a pedal assembly so that the smallest possible angle was subtended during movement through a full range of motion. Air pressure was maintained in a closed-loop between the pneumatic cylinder chamber and an air supply tank (Price et al. 1995). The system was designed so that when each muscular contraction was released, air refilled the pressurized chamber with no residual resistance from the non-pressurized chamber, actively moving the foot back to a resting position. The pneumatic cylinder/piston assembly was mounted at one end to a rigid Lexan frame and at the other end to a pedal which rotated the foot about the subject's ankle.

In the EMG protocol subjects were seated with their leg placed in the same pneumatic non-magnetic exercise ergometer as used in the MRI protocol. Repetitive plantar flexion contractions (30/minute) were performed at a load of 25% of 1-RM. The three conditions (knee angles of 0°,45°, and 90°) were completed with at least 1hr rest between conditions. The order of administration of the conditions was balanced. The set-up of the exercise ergometer was identical to that used in the MRI protocol, and subjects exercised for 2min (60 contractions) at an angular contraction velocity of ~45°/s.

EMG PREPARATION:

Pre-amplified high-impedance surface electrodes, 8mm in diameter (Therapeutics Unlimited) were placed over the belly of the SOL, MG, LG, and TA muscles with a 20mm center-to-center interelectrode distance. The ground was placed on the lateral

aspect of the knee. Signals from these electrodes were bandpass filtered (20Hz-4kHz; -3db), amplified using a differential amplifier, displayed on an oscilloscope, digitally sampled (12-bit resolution, 2000 samples per second), and stored for later analysis. The same electrodes remained in place for all three conditions (0°,45°,90°). A preliminary cross-correlation analysis revealed negligible crosstalk among these EMG channels.

EMG ANALYSIS:

The EMG signals were acquired continuously throughout the two-minute exercise protocol. The exercise ergometer (pneumatic device) and exercise performance parameters (angular contraction velocity = 45°/sec) were identical to those of the MRI protocol. During contractions the root mean square of the raw EMG signal (rmsEMG) was used to quantify the level of electrical activity for each muscle. Variations in electrode area, quantity and conductance of the gel, surface impedance, daily changes in amplifier characteristics, and temperature (Basmajian and DeLuca 1985; Lindstrom and Petersen 1981; Milner-Brown and Stein 1975) can influence EMG signal. Given the repeated measures design employed within a single test day and the balanced treatment assignment, these modifying factors were considered to be constant within each subject's testing session.

MR IMAGING:

According to the designed exercise protocol, T_2 times were compared before and after each of the three exercise sessions at 0°, at 45°, and at 90°. This data collection protocol

is different from EMG data, which was collected as electrical activity during muscular contraction, and not before and after exercise. Single slice MR imaging was performed on a 1.5-Tesla GE Signa system (General Electric, Milwaukee,WI). Subjects were positioned supine within the magnet with an extremity coil positioned midcalf and with their foot positioned in the pedal assembly of the exercise apparatus (Price et al. 1995; Price and Gore 1998). Transverse mid-calf images (10mm slice thickness; FOV=20cm: 128 × 256 matrix: NEX=1) were obtained using a multiple spin-echo sequence (TR=1000msec: TE=30,60,90,120msec; total scan time=2min 20sec). T_2 values before and after exercise were calculated in multiple regions-of-interest within the MG, LG, SOL. TA, and PER muscles. Each ROI was selected so that visible blood vessels and fat were avoided. Based upon multiple ROI's, the T_2 change was found to be uniform within each muscle tested. T_2 values were calculated by fitting four data points (4 echoes) to a mono-exponential decay using a least squares algorithm.

MR IMAGE PREPARATION AND PRESENTATION:

To generate images that would allow T_2 data to be presented as a three-dimensional color map of the lower leg, two-dimensional MR images were processed using a digital segmentation & reconstruction process. The intention of this type of presentation was to allow readers to get a more global sense of the changes in muscle activation patterns that result from altering the knee angle. Selected muscles and bones in transverse serial MRI slices taken from a representative test subject were digitally traced and shaded in cross-sections using Photoshop 5.0 (Adobe Systems, San Jose, CA). The resulting segmented

sections were stacked and reconstructed into polygonal models via a marching cubes algorithm, using Synu graphical software (National Center for Microscopy and Imaging Research at San Diego). The reconstructed muscles and bones were imported into the digital modeling and animation software 3D Studio Max (Kinetix, Milpitas, CA), in which muscles were assigned false colors according to their ΔT_2 values. A linear color scale of indigo through red (hues of 0° — 170° in 3D Studio Max HSB color space) was chosen to represent the measured range of ΔT_2 values of 0 (indigo) to 7.3 (red) at knee flexion angles of 0°, 45°, and 90°. Any negative ΔT_2 values were not significantly different from zero and were taken to represent no significant change in T_2. They were therefore assigned a value of zero. The reconstructed bones were assigned a pivot point at the position of the knee joint and the entire reconstructed leg flexed from its initial rest angle of 0° (straight), to simulate 45° and 90° bends for the purpose of illustration (Figure 2a). Two-dimensional MR images are presented as inserts in Figure 2a. These were overlaid with the same color scale as the three-dimensional reconstructions.

MRI and EMG STATISTICAL ANALYSES:

EMG amplitudes were computed for the duration of each of the 50 repetitions at each knee angle, averaged, and used for statistical analyses. Repeated measures analysis of variance (ANOVA) was used to detect significant differences among different muscles at each knee angle tested and in the same muscle at different knee angles. When significant angle × muscle differences were detected, individual ANOVA was performed to determine individual differences, followed by a Tukey's Studentized Range Test to

ascertain the source of the difference. All EMG statistical analyses were performed using the PC-SAS statistical analysis package. Values of T_2 in exercised muscles (MG, LG, SOL. PER, & AT) at rest and at 1.16min following exercise were compared in a before and after fashion using ANOVA followed by a post-hoc comparison of means (Bonferroni).

RESULTS

MRI:

Figure 1a-1d presents MR images that were acquired before and immediately after (2min) plantar flexion at 90° and 0°. Following exercise, the muscles that were recruited appear hyper-intense on these T_2-weighted images (1b & 1d). In Table 1, mean T_2 times are given at rest (before exercise) and immediately after exercise (within 2min), while figure 2a & 2b shows calculated increases in T_2 times at the three different knee angles. Following exercise at 0° T_2 times in the MG and LG increased significantly ($p \leq 0.001$,MG; $p \leq 0.001$,LG) relative to baseline T_2 times, indicating that these muscles were recruited and performed work during plantar flexion. There was no increase in the SOL. As there was no dorsi-flexion component to the exercise protocol, T_2 times in the TA did not increase. MRI was able to measure T_2 times in the peroneus (PER) observing a significant increase ($p \leq 0.02$), and suggesting that at 0° this muscle was recruited to stabilize the ankle during plantar flexion (Table 1. Figure 1d and Figure 2). At 45° T_2 times in the LG and SOL increased ($p \leq 0.05$,LG; $p \leq 0.01$,SOL) following exercise, but T_2 times in the MG were not significantly increased. However, while significance was not

established in the MG, the muscle did appear to be at least marginally activated (Table 1, Figure 2b). There were no significant increases in the TA or the PER at 45°; although, as in the MG, there did appear to be marginal activation of the PER. At 90°, only T_2 times in the SOL were significantly increased ($p \leq 0.001$) following exercise (Table 1, Figure 2b).

Fig 1 a-d

Table 1

Fig 2 a&b

EMG:

Mean amplitudes for electrical activation of the medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (SOL, and tibialis anterior (TA) are pictured in Figure 3a-3d. A repeated measures analysis of variance, incorporating the three different knee angles (0°,45°, & 90°) as a repeated measure, was conducted within each muscle. Figure 3c illustrates that there were no statistically significant differences in EMG amplitude among the three conditions for the SOL muscle ($F=0.07$, $p<0.9293$). However, the three different knee angles produced significant differences in rmsEMG amplitudes for the LG ($F=8.1$, $p<0.0001$), MG ($F=14.3$, $p<0.0001$), and TA ($F=14.3$, $p<0.0001$) amongst the three conditions (Figure 3a,b, & d). For the LG and MG, Tukey's range test revealed that EMG amplitudes for the 45° and 90° conditions were similar but significantly different from the 0° knee joint angle ($p<0.04$). For the TA, the EMG amplitudes were similar at the 0° and 45° angles, but significantly different at the 90° joint angle ($p<0.004$). Figure 3a & 3b illustrates that considerably greater EMG activity existed when the knee was

straight (0°) than when it was bent. The antagonist TA activity was small at 0° and 45°, but increased considerably at the 90° joint angle (Figure 3d).

Fig 3a-d

DISCUSSION

In this study, MRI was used to assess muscle activation in the lower leg following plantar flexion exercise performed with 0°, 45°, and 90° of knee flexion. MRI data were compared with surface EMG data of the same muscle during the same exercise. MRI and EMG scores were compared on the basis of their ability to detect qualitatively similar patterns of muscle activation. EMG and MRI both demonstrated progressively less activation of the MG and LG as the leg was flexed; however, there was less agreement between MRI and EMG in the analysis of SOL activation. In the EMG study there was considerable activation of the SOL at all knee angles, while MRI did not detect activation of the SOL when the knee was fully extended. It is likely that this discrepancy results from the different physiological phenomena that are detected by EMG and MRI. The MRI analysis unexpectedly detected activation of the PER, a muscle not targeted for study using EMG. In this study the PER is believed to have stabilized the ankle; which, because the ankle was not supported by the exercise apparatus, required increasing stabilization as the knee angle moved from 90° to 0° of flexion. Activation of stabilizing or postural muscles during voluntary contraction has been well-documented, and such muscles can begin firing even prior to the prime movers involved in the task (Lee 1980). In the TA, an antagonist of the triceps surae, the T_2 did not increase and the EMG activity

increased only during the return phase of the exercise protocol while the knee was flexed at 90° (Figure 4). This observed EMG activity at the 90° knee angle may have resulted from the design of the exercise ergometer, which allowed the ankle to move through the primary range-of-motion of dorsi-flexion in the 90° knee orientation initiating a co-contraction of the TA with the SOL. This would have resulted in a "braking-action" during the relaxation phase of each contraction/relaxation cycle (Behm and Sale 1996) that would have manifest as EMG activity in the TA. No activation of the TA was detected with MRI at any time during this study, suggesting that EMG activity in the TA at 90° was not necessarily associated with the muscle performing work.

Fig 4

The mechanisms by which physiological events initiate a change on MR images is complex, and at present are not fully understood. It is thought that exercise-induced T_2 increases are driven primarily by water shifts resulting from transient changes in tissue osmolality and perfusion (Lundvall et al. 1972; Polak et al. 1988) consequent to metabolic activation, though perfusion is not necessary for exercise-induced T_2 increases (Archer et al. 1992; Fleckenstein et al. 1988). This suggests that accumulation of tissue osmolytes, particularly intracellular lactate, may be an important factor effecting T_2 increases (Damon et al. 2002.Fleckenstein et al. 1991; Pan et al. 1991). These early studies also reported that when blood perfusion was returned to muscle tissue following exercise, there was an additional increase in T_2, suggesting that osmolality and perfusion may be additive (Archer et al. 1992; Fleckenstein et al. 1988). It is likely that exercise induced decreases in intracellular pH also plays a role in the T_2 of muscle (Damon et al. 2002).

The T_2 changes following exercise, which scale to the intensity of the work performed (Fisher et al. 1991; Price et al. 1995), are therefore sensitive to the metabolic activation required to generate force. EMG measures the wave of depolarization that initiates muscle contraction, and is sensitive to the recruitment of motor units and to the rate and patterns with which they fire. It therefore reflects the strategy employed by the nervous system to generate force or to execute a movement. The absence of T_2 changes in the presence of EMG activity suggests that although the muscle was recruited by the nervous system, it did not perform enough metabolic work to undergo a T_2 change. The relevant cases in the present study occurred at full extension, where there was EMG activity but no T_2 change in the SOL, at 90° of flexion, where EMG activity but no T_2 change was present in the TA, and at 90° where there was EMG activity in the LG and MG but no T_2 change. In the former case, this may have occurred because the contribution of the LG and MG to force production dominated that of the SOL. In the case where LG and MG activity appeared in the absence of T_2 change, this may have occurred because the bi-articular gastrocnemius was moved passively to a position on the length-tension curve at which there was little opportunity for cross-bridge cycling (Halar et al. 1978; Kawakami et al. 1998; O'Rahilly 1983). As a result, there would be less metabolic activation creating a reduced demand for ATP. The overlap of thin and thick filaments in the mono-articular SOL, conversely, is not affected by knee flexion, and so at this knee angle it must dominate the force production of plantar flexion.

By detecting selective activation of the muscles of the lower leg during plantar flexion at different knee orientations, this study demonstrates the efficacy of MRI for simultaneously detecting activation of groups of muscles. This is the first report of the

use of MRI to detect activation of the soleus during plantar flexion, and it is an essential step in the development of MRI as a tool to study the muscle activation patterns that result from complex movements. In order to be able to evaluate unknown muscle activation patterns the need exists to be able to think of different skeletal muscles as being basically similar in their MRI behavior. This study, by using MRI to examine a known movement (plantar flexion) that produces a known muscle activity pattern (Bilodeau et al. 1994; Capady and Stein 1987; Moritani and Muro 1987; Murray et al. 1976; Nardone and Schieppati 1988; Price and Gore 1998; Sloniger et al. 1998), demonstrates that MRI is a viable tool for muscle activation studies.

The current MRI and EMG data demonstrate that the relative contributions of the triceps surae muscles during plantar flexion are a shared phenomenon. The muscles of the triceps surae are utilized during plantar flexion according to knee angle and the corresponding changes in muscle length. The current EMG results are in agreement with those reported by Tomaki, et al. (1997) comparing the MG, LG, and SOL at knee angles of 0°, 30°, and 60° at speeds of 6°/sec, 30°/sec, and 60°/sec, and relative loads of 5% and 10% of 1-RM. That study reported significantly higher peak integrated EMG (IEMG) values in the SOL at 60° than at 30° and 0°. The opposite pattern was reported for the MG, with IEMG values being significantly higher at 0°. The study concluded that during plantar flexion the effect of knee angle upon muscle activation in the triceps surae was the dominant variable. Preliminary work in our laboratories has examined EMG patterns at 382°/sec observing, in agreement with Tomaki, et al. (1997), that the effect of knee angle was dominant over the contraction velocity under (unpublished observation). However, the potential effects of other factors upon lower leg muscle recruitment during plantar

flexion must not be ignored. Other studies have reported differences in muscle recruitment patterns due to fibre type content and force of contraction (Gerdle et al. 1991; Moritani and Muro 1987). In this study the results of EMG analyses of the MG, LG, and SOL muscles during plantar flexion reveal notable differences in muscle utilization patterns due to knee angle. These patterns can also be explained by noting the monoarticular structure of the SOL and the biarticular structures of the MG and LG. Since the MG and LG cross both the knee and ankle joints, their length and relative tension during plantar flexion would be affected by changes in the knee angle. Because the SOL has its origin on the soleal line of the tibia and posterior head and upper shaft of the fibula, changes in knee angle would be expected to have little effect on its function, except as it relates to its functional interaction with the MG and LG.

In summary, this study demonstrates that EMG and MRI are both capable of detecting patterns of muscle activation among the leg muscles during plantar flexion exercise. However, the results of the two techniques were not in complete agreement. This is because EMG is sensitive to the electrical activity of a muscle, while changes in T_2 are brought by metabolic activation within the muscle, pointing to potential advantages and disadvantages of each technique. Surface EMG, while rapidly responding to electrical changes in the underlying muscle fibers, can only detect the activation of those surface muscles for which it has been specifically targeted. Inasmuch as the surface EMG signal is biased by the activity of more superficially-oriented muscle fibers (Knight and Kamen 2000), MRI changes, which respond more slowly to changes in muscle activation, are able to detect the metabolic activation of deeper muscle fibers and possibly other muscles. The two techniques may therefore be viewed as complementary, rather than

competitive. The plantar flexion activation patterns detected by EMG agree with those reported in other studies (Bilodeau et al. 1994; Capady and Stein 1987; Moritani and Muro 1987; Murray et al. 1976; Nardone and Schieppati 1988; Price and Gore 1998; Sloniger et al. 1998). The relative agreement between MRI and EMG observed in this study suggests that MRI is a valid tool for studying muscle activation. From these data, we conclude that muscle activation during plantar flexion is a shared phenomenon, with the degree of contribution of the plantar-flexor muscles being influenced by the degree of flexion of the knee joint. We further conclude that this phenomenon is measurable by both EMG and MRI.

ACKNOWLEDGEMENTS

This research was supported by a grant from the United States Public Health Service: RO1 DK-49230. Dr. Thomas B. Price is an investigator for the U.S.Army (DAMD17-96-C-6097). All experiments complied with approved Human Use Protocols at the respective universities where subjects were studied. All university Human Use Protocols are required to comply with the current federal laws governing human research in the USA.

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KNEE-ANGLE	STATUS	MG T_2 [msec]	LG T_2 [msec]	SOL T_2 [msec]	PER T_2 [msec]	TA T_2 [msec]
90°	REST	29.4±0.4	29.5±0.4	31.1±0.4	29.7±0.5	29.2±0.8
	EXE.	29.7±0.5	29.6±0.5	34.3±0.5**	30.7±0.4	27.9±0.5
45°	REST	30.0±0.6	30.0±0.6	31.1±0.5	30.4±0.8	28.0±0.4
	EXE.	32.3±1.0	32.5±1.0†	34.2±0.8*	32.4±1.0	28.6±0.8
0°	REST	30.5±0.7	30.9±0.8	31.1±0.7	30.9±0.9	29.9±1.8
	EXE.	37.8±1.1**	38.2±1.1**	31.4±0.9	36.0±1.7†	29.4±1.0

Table 1: Mean T_2 ± SE before and after 2min plantar flexion exercise at 0°, 45°, and 90° knee flexion. Comparisons are made within each muscle, between resting and post-exercise T_2 times: † $p \leq 0.05$, * $p \leq 0.01$, ** $p \leq 0.001$. MG = medial gastrocnemius, LG = lateral gastrocnemius, SOL = soleus, PER = peroneus, TA = tibialis anterior.

FIGURE LEGEND

Figure 1a-d: MRI of the lower leg (mid-calf) obtained before and immediately after exercise. A: Before plantar flexion with the knee flexed at 90°, B: After plantar flexion with the knee flexed at 90°. C: Before plantar flexion with the knee fully extended (0° flexion), D: After plantar flexion with the knee fully extended (0° flexion). At full knee extension the peroneus was recruited to stabilize the ankle.

Figure 2a & 2b: Plantar flexion induced change in transverse relaxation (T_2) times within the medial gastrocnemius (MG), the lateral gastrocnemius (LG), the soleus (SOL), and the peroneus (PER) at 0°, 45°, and 90° of knee flexion. The tibialis anterior (TA) was not significantly recruited at any of the knee angles tested. 2a. Data are presented as a color image that is a three-dimensional representation of a series of two-dimensional MR images. Colors are related to a scale of T_2 increases that are a direct representation of the degree of metabolic activation of the muscle. MRI typically records exercise-induced T_2 increases as a global change in all MR slices through the muscle; therefore, colors are presented as the same throughout the muscle. Inserts are color overlays onto two-dimensional MR images. 2b. The same data presented as a bar graph. * $p \leq 0.05$ resting muscle versus muscle post-exercise. Values are mean ± SEM.

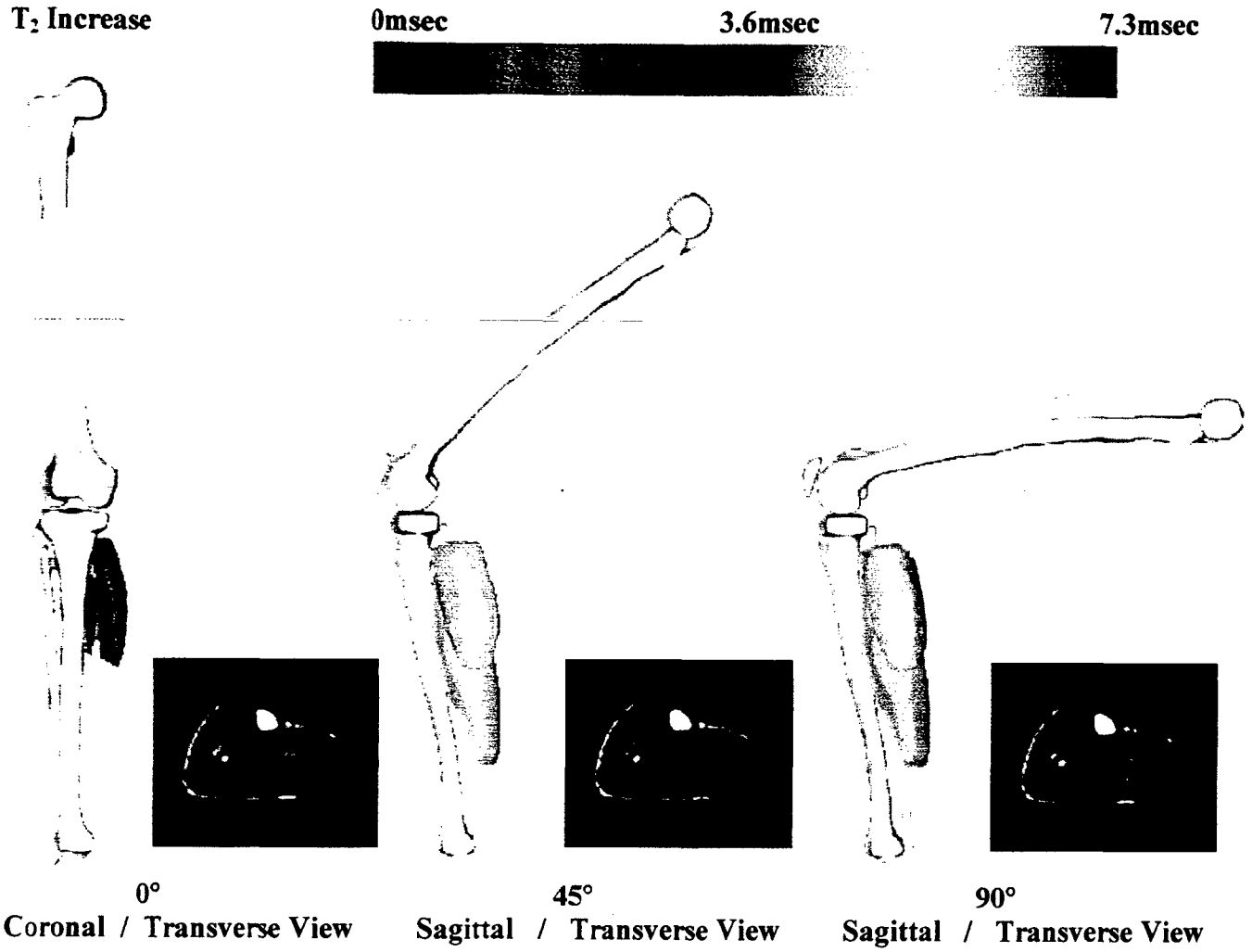
Figure 3a-d: Plantar flexion induced MRI T_2 changes [msec] and EMG activation [μV] of the MG, the LG, the SOL, and the TA at 0°, 45°, and 90° of knee flexion. 3a. MG T_2

changes MRI and EMG activation are greater at 0° than at 45° and 90° (* $p<0.006$). MRI and EMG are greater at 45° than at 90° (** $p<0.04$). 3b. LG T_2 changes MRI and EMG activation are greater at 0° than at 45° and 90° (* $p<0.04$). 3c. SOL T_2 changes MRI is less at 0° than at 45° and 90° (* $p<0.0003$) but not significantly different for EMG. 3d. TA EMG activation was greater at 90° than at 0° and 45° (*** $p<0.004$) but not significantly different for MRI. Negative values for TA MRI were not significantly different from zero. All values (3a-d) are mean ± SEM.

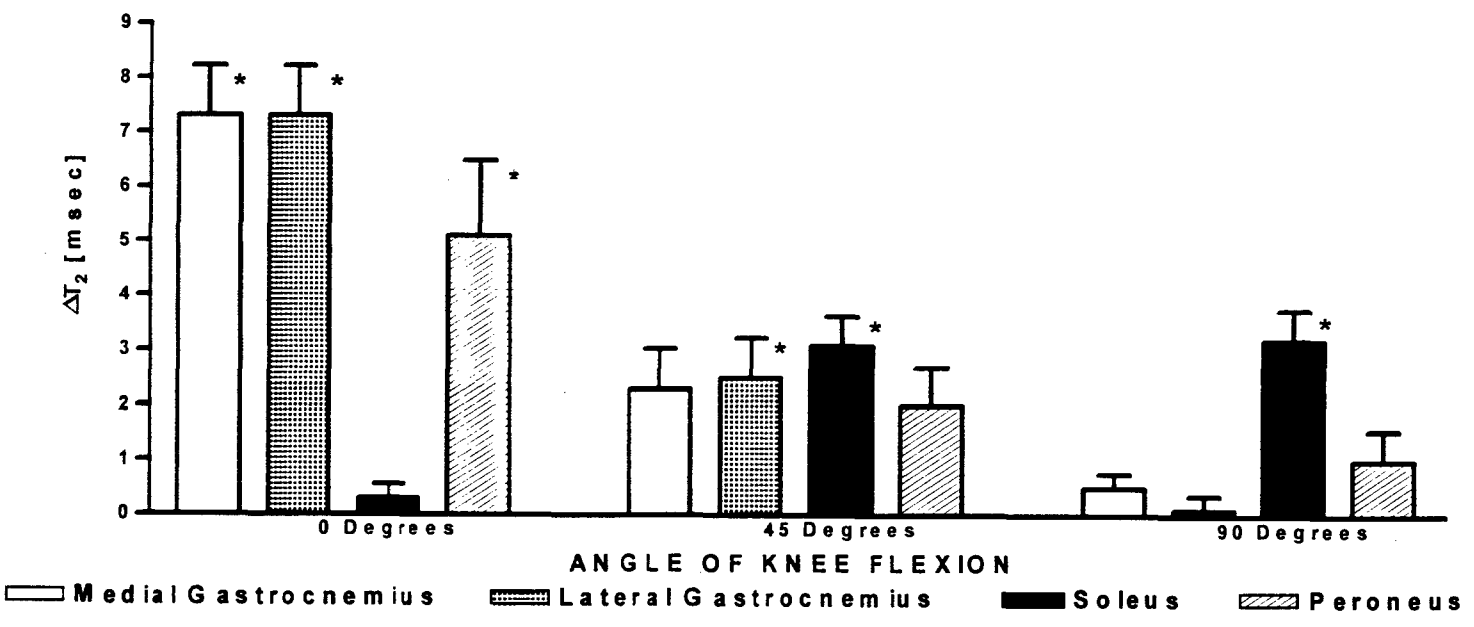
Figure 4: Two and a half seconds of myoelectric activity at 90° of knee flexion from: (A) the soleus and (B) the tibialis anterior of an individual subject. Unlike the other knee orientations (0° and 45°, data not shown), at 90° the behavior of these two muscles was different. While there were soleal plantar-flexor bursts at 0.5sec, 1.5sec, and 2.5sec following the initiation of the contraction, there was very little tibialis anterior activity. During the relaxation phase (at 0sec, 1sec, and 2sec), tibialis anterior was active and co-contracting with the soleus.

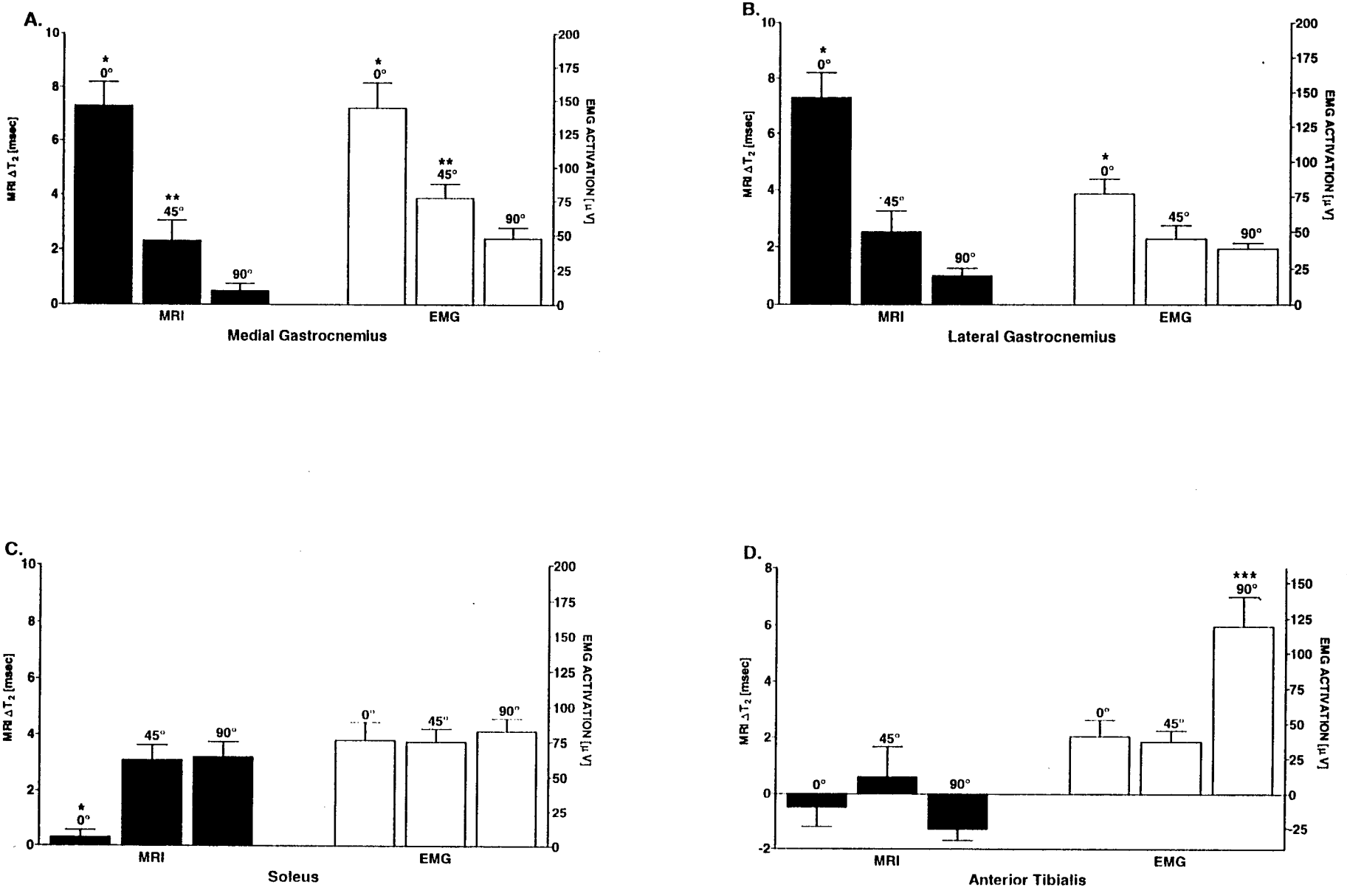
C
A
D
B

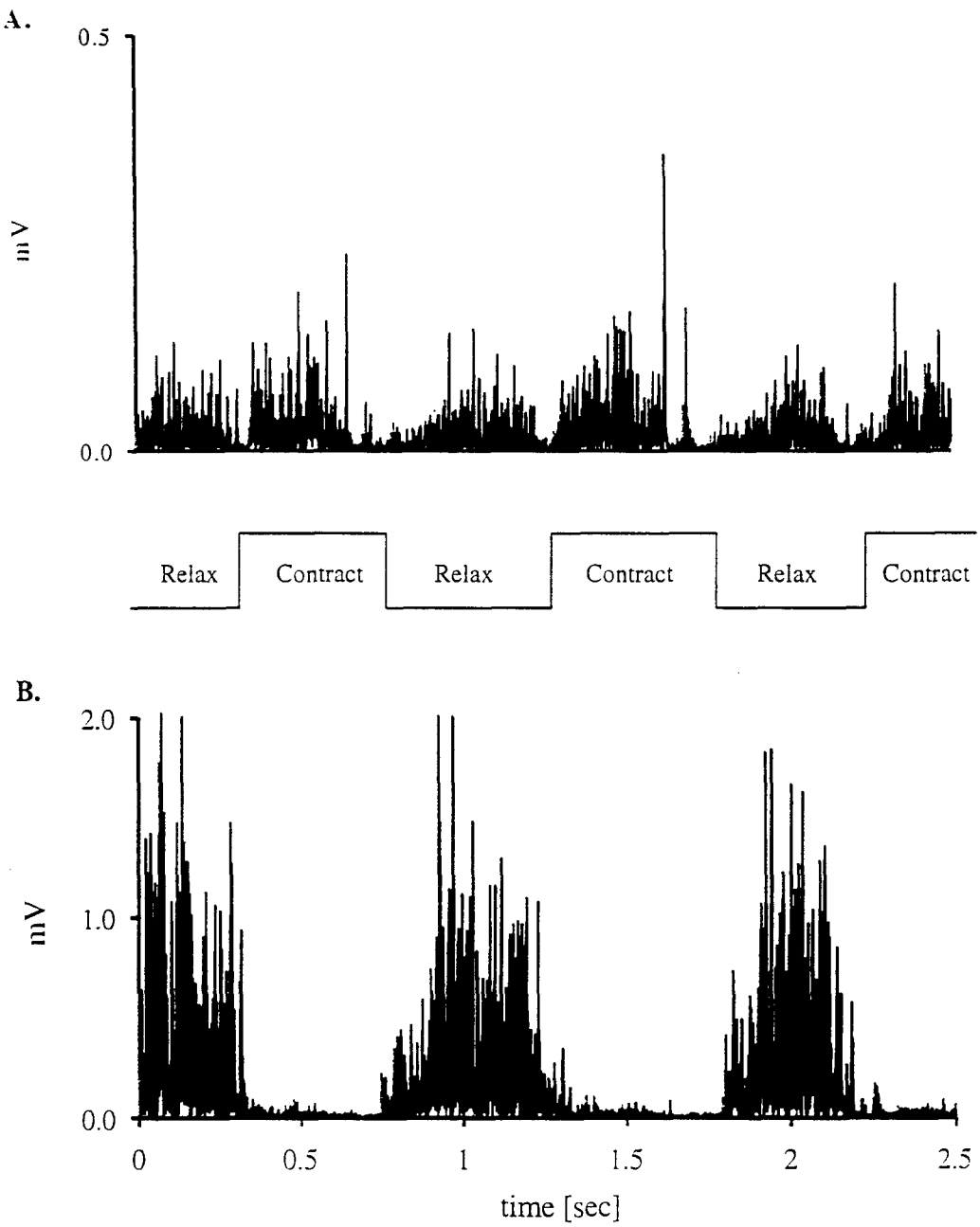
A:


T2 Increase
0msec
3.6msec
7.3msec
0°
Coronal / Transverse View
45°
Sagittal / Transverse View
90°
Sagittal / Transverse View

B:


ΔT2 [msec]
0 Degrees
45 Degrees
90 Degrees
ANGLE OF KNEE FLEXION
Medial Gastrocnemius
Lateral Gastrocnemius
Soleus
Peroneus


A.
MRI ΔT2 [msec]
EMG ACTIVATION [μV]
*
0°
**
45°
90°
*
0°
**
45°
90°
MRI
EMG
Medial Gastrocnemius
B.
MRI ΔT2 [msec]
EMG ACTIVATION [μV]
*
0°
45°
90°
*
0°
45°
90°
MRI
EMG
Lateral Gastrocnemius
C.
MRI ΔT2 [msec]
EMG ACTIVATION [μV]
*
0°
45°
90°
0°
45°
90°
MRI
EMG
Soleus
D.
MRI ΔT2 [msec]
EMG ACTIVATION [μV]
0°
45°
90°
0°
45°
***
90°
MRI
EMG
Anterior Tibialis


A.
0.5
mV
0.0
Relax
Contract
Relax
Contract
Relax
Contract
B.
2.0
1.0
mV
0.0
0
0.5
1
1.5
2
2.5
time [sec]

Early post-exercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement

Running Head: Nutritional Supplementation Following Exercise

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ABSTRACT

In the present study, we tested the hypothesis that a carbohydrate-protein (CHO-PRO) supplement would be more effective in the replenishment of muscle glycogen after exercise compared to a carbohydrate supplement of equal carbohydrate content (LCHO) or caloric equivalency (HCHO). Following 2.5±0.1 hours of intense cycling to deplete the muscle glycogen stores, subjects (n = 7) received, using a rank ordered design, a CHO-PRO (80g CHO, 28g PRO, 6g fat), LCHO (80g CHO, 6g fat) or HCHO (108g CHO, 6g fat) supplement immediately (10min) and 2 hours post exercise. Before exercise and during 4 hours of recovery, muscle glycogen of the vastus lateralis was determined periodically by nuclear magnetic resonance spectroscopy. Exercise significantly reduced the muscle glycogen stores (40.9±5.9 mmol/L CHO-PRO, 41.9±5.7 mmol/L HCHO, 40.7±5.0 mmol/L LCHO mmol/L, final concentrations). Following 240 min of recovery, muscle glycogen was significantly greater for the CHO-PRO treatment (88.8±4.4 mmol/L) when compared with the LCHO (70.0±4.0 mmol/L; p=0.004) and HCHO (75.5±2.8 mmol/L; p=0.013) treatments. Glycogen storage did not differ significantly between the LCHO and HCHO treatments. There were no significant differences in the plasma insulin responses among treatments although plasma glucose was significantly lower during the CHO-PRO treatment. These results suggest that a CHO-PRO supplement is more effective for the rapid replenishment of muscle glycogen after exercise than a carbohydrate supplement of equal carbohydrate or caloric content.

Key words: catecholamines, glucose, lactate, insulin, NMR spectroscopy, glycogen, exercise

INTRODUCTION

Muscle glycogen is an essential fuel source for moderate to high intensity exercise. Once depleted the capacity to perform at these exercise intensities is lost or severely limited (1, 2, 9, 11). Therefore, the faster the muscle glycogen stores can be replenished following exercise the faster the recovery process and theoretically the greater the return of performance capacity. In recent years, different methods of rapidly increasing the muscle glycogen stores have been extensively investigated. Research studies addressing this question have focused on the timing (13,18), frequency (10), amount of supplementation (3, 12, 14, 15), as well as the type of supplement to ingest (6, 21, 24, 30. 32). With regard to the type of supplement, Zawadzki et al. (32) found that the combination of carbohydrate and protein was more effective than carbohydrate alone in the replenishment of muscle glycogen during the 4 h immediately after exercise. It was suggested that this greater rate of muscle glycogen storage was the result of a greater plasma insulin response when the carbohydrate-protein supplement was provided. However, more recent studies have taken issue with the benefits of adding protein to a carbohydrate supplement (7, 15, 27, 29, 30). Results from these studies suggested that the enhancement in muscle glycogen storage found in the study by Zawadzki et al (32) was simply due to a greater amount of calories provided during the carbohydrate-protein treatment as compared to the carbohydrate treatment. Moreover, it was suggested that if adequate carbohydrate were provided, the addition of protein would have no beneficial effect on muscle glycogen recovery (15). It should be noted, however, that the carbohydrate supplement used by Zawadzki et al. (32) had previously been shown to maximize muscle glycogen storage during recovery when provided immediately post exercise and at 2 h intervals thereafter (3, 12, 14). Thus, it is possible that significant differences in experimental designs and supplement compositions could possibly account for the lack of agreement among studies.

In the present study, we re-evaluated the potential of a carbohydrate-protein supplement to enhance muscle glycogen storage after vigorous exercise. We employed

natural abundance ^{13}C nuclear magnetic resonance (NMR) spectroscopy to measure glycogen concentrations primarily in vastus lateralis, with some small contribution from vastus medialis and rectus femoris. ^{13}C NMR provides the non-invasive and continuous assessment of muscle glycogen concentrations and their dependence on diet and exercise. With muscle biopsies, the number and frequency of measurements and the sampling of only a small volume in a non-homogeneous tissue have been a limitation. Because of the non-invasive nature of the NMR technique (22), we were able to measure glycogen with better time resolution (frequency), repeatability, and precision during recovery than previously employed in biopsy studies (22). We hypothesized that: 1) this increase in time resolution would enable us to detect subtleties in post exercise glycogen recovery that would not otherwise be available, and 2) a carbohydrate-protein supplement would increase the rate of muscle glycogen storage compared to a carbohydrate supplement of equal carbohydrate or caloric content.

METHODS

Subjects. Seven trained male cyclists were studied. Subjects were 23±1 years of age (19-26y), 181±1 cm in height (178-183cm), and weighed 74±2 kg (70-82kg). Subjects were screened by an interview for medical history and had no family history of diabetes, hypertension, or metabolic disorder. There maximum oxygen uptakes (VO_{2max}) and maximum heart rates (HR_{max}) were measured using an expired gas analyzer (SensorMedics Vmax 29, Yorba Linda, CA) and Polar © heart monitor (Polar Electro Oy, Finland), at least 72 h prior to the beginning of the study. VO_{2max} and HR_{max} were 61.1±2.1 ml/kg-min (50.6-66.4 ml/kg-min) and 190±4 bpm (178-201 bpm), respectively. Resting heart rates were 44±2 bpm (40-50 bpm). Resting quadriceps glycogen concentrations were 147.0±6.6 mmol/l (84.6-210.1 mmol/l). Subjects gave informed consent to participate in this study according to a protocol approved by the Human Investigation Committee of the Yale University School of Medicine.

Experimental Protocol. Subjects participated in the study on three separate occasions spaced at least one month apart. Subjects were rank-ordered according to VO_{2max} and assigned treatments according to a predetermined counter-balanced design to reduce any sequence effects. Subjects arrived at the Yale University School of Medicine General Clinical Research Center (GCRC) at 6:00 PM on the evening before each study. Subjects were given a mixed meal (50 % carbohydrate, 30 % fat, 20 % protein) 12 h before beginning the exercise protocol. During the 12 h immediately preceding exercise, subjects remained in the GCRC where they were monitored and consumed only water. At 6:00 AM on the morning of the study, following the overnight fast and at least 30 minutes before any samples were collected, a Teflon catheter was inserted in an antecubital vein to obtain blood samples. To establish resting glycogen levels in the vastus lateralis, a natural abundance ^{13}C NMR scan was performed at the mid-thigh using the methods described below. Baseline blood samples were also obtained during this period. Subjects then performed the exercise protocol. A blood sample and a single ^{13}C NMR scan were obtained immediately upon cessation of exercise. Following the NMR scan, 10 min into the recovery period, subjects were given the first of two, 472ml (16 oz) post-exercise nutritional supplements. During the remainder of the first hour of recovery ^{13}C NMR scans were performed at 20, 40 and 60 min, and blood samples were collected at 30 and 60 min. At 120 min into the recovery period subjects were given the second 472ml dose of the nutritional supplement. Additional ^{13}C NMR scans were performed at 120, 180, and 240 min of recovery. A total of seven ^{13}C NMR measurements of quadriceps glycogen were obtained during the 240 min initial recovery period. Blood samples were collected at 120, 150, 180, 210, and 240 min of recovery. Following 240 min of recovery subjects were given a mixed meal and released from the GCRC.

Exercise Protocol. Following baseline NMR and blood measurements, subjects exercised on their own bicycles equipped with stationary adapters. Each subject performed 2 h of cycling at 65-75 % of his VO_{2max}. Oxygen uptake was measured every 30 min and

workload adjusted accordingly. After 2 h of cycling subjects performed a series of 1 min sprints at maximum effort. Each sprint was separated by 1 min of rest during which the subject cycled at a self-selected leisurely rate. This sprint phase of the exercise protocol was maintained until the subject's plasma glucose level had dropped below 3.89 mmol/l. This was to insure that liver glycogen stores were depleted to the same degree during each trial thus reducing variability in carbohydrate availability during recovery. There were no significant differences in the total number of sprints completed between the three occasions that each subject performed the exercise protocol. The mean number of sprints completed was 15±2.

Nutritional Supplementation. Subjects were studied on three separate occasions, performing identical exercise protocols each time. In each study subjects were given one of three different nutritional supplements immediately following exercise (within 10 min of cessation) and again 2 h after cessation of exercise. The three different nutritional supplements were: carbohydrate + protein (CHO-PRO), a commercial product (Systems GO International) containing 378 kcal [240 kcal (80g) of carbohydrate + 84 kcal (28g) of protein + 54 kcal (6g) of fat]; isocaloric carbohydrate (HCHO): 378 kcal [324 kcal (108g) of carbohydrate + 54 kcal (6g) of fat]; and isocarbohydrate (LCHO): 294 kcal [240 kcal (80g) of carbohydrate + 54 kcal (6g) of fat]. The nutritional supplements were delivered in liquid form (472 ml) at the two separate time points, for a total of 944 ml (32 fluid ounces) over the 4 h recovery period. Total caloric intake for the CHO-PRO and HCHO supplements was 756 kcal, and for LCHO caloric intake was 588 kcal. Because the CHO-PRO supplement included a small amount of fat (3g/236ml) for flavor, subjects assigned the HCHO and LCHO treatments consumed 3g of clarified warm butterfat, followed immediately by the aqueous CHO portion of the treatment. The ratio of simple to complex CHO of all treatments was identical. This was achieved by making flavored aqueous solutions of HCHO and LCHO using the same formula of sucrose and maltodextrin, as the commercial CHO-PRO supplement. We opted to use an available CHO-PRO supplement

because its percentage of carbohydrate to protein was similar to what was employed in the Zawadzki et al. (32) study, and because it was thought to contain a sufficient amount of protein for our purpose.

NMR Spectroscopy. Natural abundance ^{13}C NMR spectroscopy was performed at 2.1T (Tesla) on a Bruker Biospec spectrometer with a 100 cm-diameter magnet bore. During the measurements, subjects remained supine within the magnet with a surface coil radio frequency (RF) probe resting mid-thigh, directly above the vastus lateralis. Power deposition profiles indicate that the majority of NMR signal was received from the vastus lateralis, with some small contribution (<10%) from the vastus medialis and the rectus femoris. During the data acquisition period RF power was pulsed through the surface coil at a frequency of 22.5 MHz (^{13}C resonance frequency). A 9 cm diameter circular ^{13}C surface coil RF probe was used for spectral acquisitions. Shimming, imaging, and ^{1}H decoupling at 89.5 MHz was performed with a 12 cm x 12 cm series butterfly coil. Proton linewidths are typically shimmed to < 70 Hz. A microsphere containing a ^{13}C labeled formate was fixed at the center of the RF coil for calibration of RF pulse widths. Subjects were positioned by an image-guided localization routine that employs a T_1-weighted gradient-echo image (repetition time (TR)=82msec, echo time (TE)=21msec). Subjects' thighs were positioned so that the isocenter of the magnetic field was ~2 cm into the vastus lateralis muscle. By determining the 180° flip angles at the center of the observation coil from the microsphere standard, RF pulse widths were set so that the 90° pulse was sent to the center of the muscle. This technique maximizes suppression of the lipid signal that arises from the subcutaneous fat layer and optimizes signal from the muscle. The ^{1}H decoupled ^{13}C RF pulse sequence has been designed so that 5472 -^{13}C transients are obtained. The repetition time for ^{13}C acquisition was 87 msec, and ^{1}H continuous wave decoupling was truncated to 25 msec at the beginning of each ^{13}C acquisition to prevent excessive RF power deposition in the muscle. Power deposition, assessed by magnetic

vector potential specific absorption rate (SAR) (5), was calculated at < 4 W/kg. The total scan time for each spectrum was 8 minutes.

Blood Sampling. Venous blood samples were assayed for glucose, lactate, insulin, catecholamines, and free fatty acids (FFA). Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Fullerton, CA.) (17). Lactate was also assayed by an enzymatic method (8). Plasma insulin (26) and catecholamines (23) were assayed by a double-antibody radioimmunoassay technique. FFA were assayed by a microfluorometric method (19).

Statistical Analysis. Statistical analyses were performed using SPSS software (SPSS, Chicago, IL). Values are reported as means ± SE. For muscle glycogen analysis, a two-way ANOVA (treatment x time) with repeated measures was employed to identify significant major effects. Significant differences among means were determined by pair-wise comparisons using estimated marginal means. The blood and plasma samples were analyzed in the same manner as the muscle glycogen except that the samples drawn during exercise were analyzed separately from those drawn during recovery. Statistical significance was set at $p \leq 0.05$.

RESULTS

Muscle glycogen:

There were no significant differences in muscle glycogen concentration among the three treatments before (141.8±15.4 mmol/L CHO-PRO, 150.8±9.5 mmol/L HCHO, 148.5±9.8 mmol/L LCHO) or immediately following exercise (40.9±5.9 mmol/L CHO-PRO, 41.9 mmol/L HCHO, 40.7 mmol/L LCHO mmol/L). Figure 1 illustrates the pattern of glycogen storage for each treatment during the 4 h recovery period. Total glycogen storage at 4 h was significantly greater during the CHO-PRO treatment compared to the HCHO and LCHO treatments (Figure 2). There was no difference in glycogen storage between the HCHO and LCHO treatments. In the CHO-PRO treatment the difference in

total glycogen storage resulted from a greater rate of glycogen storage during the first 40 min of recovery and a more sustained rate of glycogen storage during the final 2 h of recovery (Figure 1 and 3). Of the total glycogen utilized during exercise, the amount recovered in the first 40 min was 22% for the CHO-PRO treatment, but only 11.5 % and 5.5% for the HCHO and LCHO treatments, respectively. Between 40 and 120 min of recovery the rate of glycogen restoration declined during the CHO-PRO and HCHO treatments, but accelerated during the LCHO treatment. After 120 min of recovery, glycogen restoration was 30.4% during the CHO-PRO treatment, 23.9% during the HCHO treatment and 23.0% for the LCHO treatment. Following the 2 h supplement, the rate of glycogen storage increased again with the CHO-PRO treatment. This secondary increase in glycogen storage did not occur during the HCHO or LCHO treatments. After 4 h of recovery, 46.8% of the glycogen utilized during exercise had been replenished with the CHO-PRO treatment, 31.1% with the HCHO treatment and 28.0% with the LCHO treatment.

Plasma Metabolites and Hormones:

Lactate: Plasma lactate rose significantly during exercise, but was not different among the 3 treatments (Figure 4a). During recovery, however, plasma lactate was significantly lower during the CHO-PRO treatment compared to the HCHO and LCHO treatments at 30, 60, 180, 210 and 240 min. There were no differences in plasma lactate between the HCHO and LCHO treatments at any time point during recovery.

Insulin: Plasma insulin levels did not differ at any time among treatments (Figure 4b). After ingestion of the first supplement, insulin levels rose significantly and then reached a plateau until the second supplement was ingested. Insulin levels then increased during the next hour and declined steadily thereafter.

Glucose: Plasma glucose declined during exercise below 3.89 mmol/l for each treatment (Figure 4c). Within 30 min after ingestion of the first supplement, blood glucose increased significantly regardless of the treatment ingested. However, the increase was

significantly greater after the HCHO and LCHO treatments than after the CHO-PRO treatment. Blood glucose during the HCHO treatment remained significantly elevated above the CHO-PRO treatment through the first 3 h of recovery. Similar results were found when comparing the CHO-PRO and LCHO treatments, except that blood glucose was not different between these treatments at 120 min of recovery.

Free fatty acids (FFA). No differences in plasma FFA occurred during exercise or recovery for the three treatments (Figure 5a). Plasma FFA increased significantly during exercise. They declined precipitously during the first 60 min of recovery and then continued declining at a slower rate, reaching baseline values by 180 min of recovery.

Epinephrine and norepinephrine. The catecholamine responses during exercise and recovery were similar for each treatment. However, the response between epinephrine (Figure 5b) and norepinephrine (Figure 5c) differed. Plasma levels of both epinephrine and norepinephrine increased during the first 70 min of exercise. Epinephrine continued to rise during the final 60 min, but during this time the plasma concentration of norepinephrine declined. During recovery there was a rapid decline in epinephrine back to baseline values within 30 min, while norepinephrine approached baseline more slowly.

DISCUSSION

In the present study, the addition of protein to a carbohydrate supplement (CHO-PRO) yielded significantly greater muscle glycogen storage in the 4 h immediately after heavy exercise, as compared with carbohydrate supplements containing either an equal weight of carbohydrate (LCHO) or an equal total caloric content (HCHO). There was no significant difference in the muscle glycogen storage between the HCHO and LCHO supplements. The percentage of glycogen restored during the 4 h recovery period was 46.8%, 31.1% and 28.0% for the CHO-PRO, HCHO and LCHO treatments, respectively.

The finding that a carbohydrate-protein supplement enhanced the storage of muscle glycogen when compared to a carbohydrate supplement of equal weight of carbohydrate is

in agreement with the early research of Zawadzki et al. (32) and the more recent research of van Loon et al. (30). Furthermore, the current results reveal that a carbohydrate-protein supplement may be more effective for the restoration of muscle glycogen than a carbohydrate supplement of equal caloric content. Due to the non-invasive nature on the ^{13}C NMR method for glycogen determination, we were also able to dramatically increase the time resolution of the glycogen measurements. The enhanced time resolution revealed subtle effects of different nutritional supplements on muscle glycogen recovery that have not been previously observed. From this additional data we found that ingestion of a CHO-PRO supplement promptly after severe exercise enhances glycogen synthesis during the initial minutes (0-40 min) of recovery, and that muscle glycogen recovery is further enhanced when a second CHO-PRO supplement is consumed 120 min into the recovery period. This enhancement was not observed with either the HCHO or the LCHO supplements.

Zawadzki et al. (32) found that muscle glycogen storage during the 4 h immediately after exercise was increased by 38% if protein was added to a carbohydrate supplement of equal carbohydrate content. The supplements were provided immediately and 2 h after exercise with the carbohydrate-protein supplement consisting of approximately 1.6 g carbohydrate and 0.6 g protein /kg body wt and the carbohydrate supplement consisting of 1.6 g carbohydrate/kg body wt. However, van Hall et al. (28, 29) could find no difference in post exercise muscle glycogen storage when comparing supplements similar to those used by Zawadzki et al. (32). Moreover, van Hall et al. (29) reported that leg glucose uptake was similar during recovery for the carbohydrate and carbohydrate-protein supplements.

Recently, van Loon et al. (30) compared the effects of a carbohydrate-protein supplement with carbohydrate supplements containing either an equal weight of carbohydrate or caloric equivalency. They reported that with the addition of a protein-amino acid mixture to a supplement of 0.8g carbohydrate/kg body wt/h increased the rate of

glycogen storage by more than 100% above that produced by an equivalent carbohydrate supplement. However, they also reported that glycogen storage was similar when comparing a carbohydrate plus protein-amino acids supplement with a carbohydrate supplement of equal caloric content. Tarnopolsky et al. (27) and Carrithers et al. (7) also found no difference in muscle glycogen storage during the initial hours of recovery when supplements containing carbohydrate or carbohydrate-protein of equal caloric content were compared.

Considering the research that has been presented, it appears that the addition of protein to a carbohydrate supplement will increase the rate of muscle glycogen storage during the hours immediately after exercise if the supplement contains a low to moderate amount of carbohydrate. What is less evident is whether the advantage of a protein-carbohydrate supplement relative to muscle glycogen storage is maintained when compared with a carbohydrate supplement of equal caloric content. There are several possible explanations accounting for the contrasting results among studies. For example, Tarnopolsky et al. (27) and Carrithers et al. (7) supplemented with lower protein concentrations than used in the present study. There were also differences in the frequency of supplement administration among studies. In the present study, supplements were provided immediately after and 2 h after exercise. In the studies that found no difference in glycogen storage among isocaloric supplements, supplements were provided every 15 or 30 min (7, 15, 27, 30). Large doses of carbohydrate provided at frequent intervals such as every 15 min have been reported to promote glycogen storage rates considerably higher than those seen when supplementing at 2 h intervals (10, 21). Thus supplementing with smaller doses, but more frequently, could alter the rate of absorption of carbohydrate and protein and possibly limit the advantage of the protein.

The time allotted for glycogen to recover may also yield different results. In the present study, differences in muscle glycogen between the CHO-PRO treatment and HCHO treatment occurred only after 40 min and 4 h of recovery. Therefore, detection of a

treatment effect may also depend on design differences in the recovery protocol. This possibility is particular relevant when considering the negative finding of van Hall et al. (28). These investigators reported that glycogen storage rates during 3 h of recovery, although not statistically significant, were approximately 20% higher following supplementation with either a carbohydrate and whey or wheat protein hydrolysate supplement as compared with supplementing with a comparable carbohydrate supplement. Supplements were provided at 1 h intervals. The results are similar to those in the present study, and raise the possibility that a significant difference in glycogen storage might have been detected if the recovery period had been extended an additional hour.

The extent to which the addition of protein to a large carbohydrate supplement could increase the rate of post exercise glycogen storage was recently addressed by Jentjens et al. (15). They provided 1.2 g of carbohydrate/kg body wt/h with and without a protein-amino acid mixture. Supplements were provided immediately after exercise and every 30 min thereafter. No difference in the rate of glycogen storage was found between treatments. Jentjens et al. (15) concluded that when the total carbohydrate in a supplement is very high the addition of a protein-amino acid mixture does not further increase the rate of muscle glycogen storage, i.e., the rate of glycogen storage can be maximized if sufficient carbohydrate is provided. Although the present study was not designed to address this question directly, it should be noted that glycogen storage was similar for the HCHO and LCHO treatments and that this finding is in agreement with earlier research (3, 12, 14). Our results, therefore, suggest that the addition of protein to a carbohydrate supplement can in fact raise the rate of muscle glycogen storage beyond the maximal rate produced by carbohydrate alone. The ability of protein to maximize the carbohydrate response, however, may be restricted to conditions when supplements are provided at intervals of 2 h our greater. As mentioned previously, providing carbohydrate supplements at more frequent intervals appears to increase their effectiveness and thus render the addition of protein to the supplement less effective (10, 21).

Results from several studies have suggested that the rate of muscle glycogen storage after carbohydrate supplementation is related in part to the plasma insulin response (24, 30, 32). Thus, the rationale for adding protein to a carbohydrate supplement has been to increase the effectiveness of the supplement toward raising the plasma insulin concentration (30, 32). In the present study the increased glycogen storage during the CHO-PRO treatment could not be attributed to a greater insulin response, nor could it be attributed to differences in plasma catecholamines or circulating FFA levels, both of which can antagonize the action of insulin (4, 16, 20, 25). It was observed, however, that during recovery plasma glucose and lactate were lower during the CHO-PRO treatment than during the HCHO or LCHO treatments. This observation might indicate an increase in plasma glucose uptake and a redistribution of intracellular glucose disposal by the addition of protein to a carbohydrate supplement. Supporting this possibility is the finding of Yaspelkis and Ivy (31) that the addition of arginine to a carbohydrate supplement lowered glucose oxidation during recovery while it tended to increase the rate of muscle glycogen storage.

An important observation in the present study was the rapid increase in muscle glycogen storage during the first 40 min of recovery as a result of the CHO-PRO treatment. Glycogen storage rates with the CHO-PRO treatment were 2 to 4 times faster than with the HCHO and LCHO treatments, and accounted for approximately 50% of the glycogen stored during the 4 h CHO-PRO recovery period. Glycogen storage for the HCHO and LCHO treatments did not reach the level obtained during the first 40 min of the CHO-PRO treatment for 2 h. These results have practical implications and suggest that a CHO-PRO supplement would be very advantageous when recovery time is extremely limited.

In conclusion, the present results suggest that a distinct advantage in muscle glycogen storage can be achieved after exercise with the addition of protein to a carbohydrate supplement. When supplementation occurs immediately post and 2 h post exercise, this advantage appears to be maintained even when compared with a carbohydrate

supplement of equal caloric content. The increased rate of muscle glycogen storage following carbohydrate-protein supplementation does not appear to be due to an enhanced plasma insulin response and is most evident during the first 40 min of recovery. This later finding would suggest that a CHO-PRO supplement might be most beneficial during short recovery periods. CHO-PRO supplementation for exercise recovery might also be advantageous if minimizing carbohydrate consumption is necessary or a personal preference such as during a weight management program.

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ACKNOWLEDGEMENTS

This study was supported by grants from the US Army (DAMD17-96-C-6097) and Systems Go International, Tampa, FL. GCRC support was provided with the support of a grant from the National Institutes of Health (M01RR 00125).

FIGURE LEGENDS

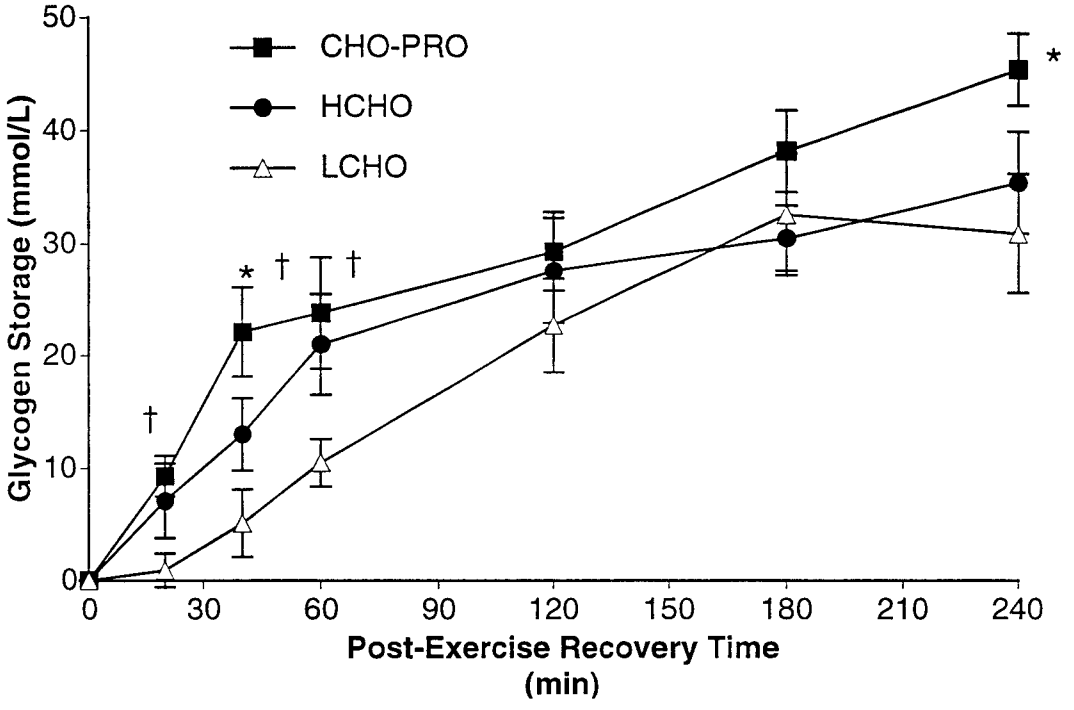
Figure 1. The patterns of muscle glycogen storage during recovery as determined by nuclear magnetic resonance spectroscopy for the carbohydrate-protein (CHO-PRO), isocarbohydrate (LCHO) and isocaloric carbohydrate (HCHO) supplements. Supplements were provided immediately after and 2 h after exercise. * PRO-CHO significantly different than LCHO and HCHO. † PRO-CHO significantly different than LCHO. ¶ HCHO significantly different than LCHO.

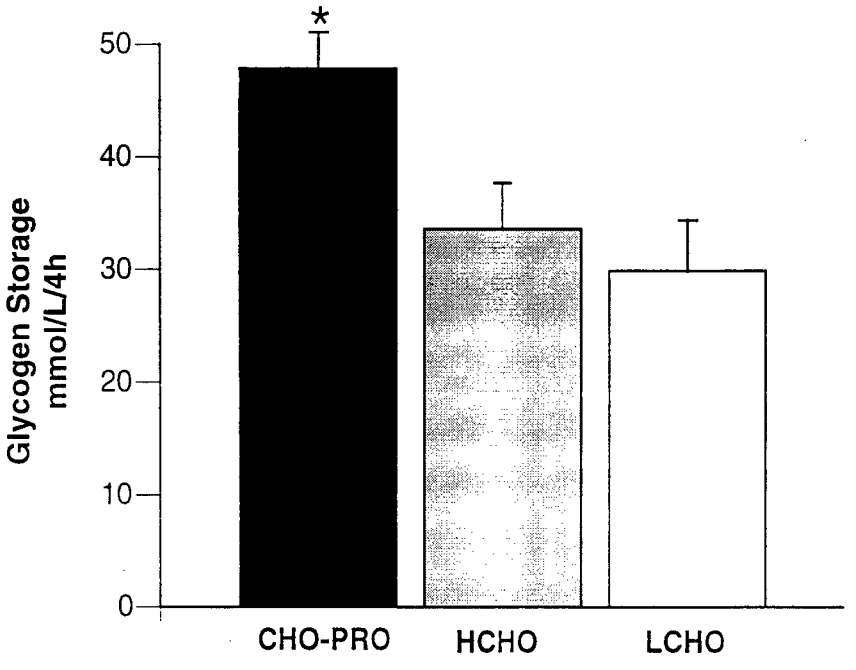
Figure 2. Total muscle glycogen storage in the vastus lateralis during 4 h of recovery from intense cycling. Treatments were carbohydrate-protein (CHO-PRO), isocarbohydrate (LCHO) and isocaloric carbohydrate (HCHO) supplements provided immediately after and 2 h after exercise. * Significantly different than HCHO and LCHO.

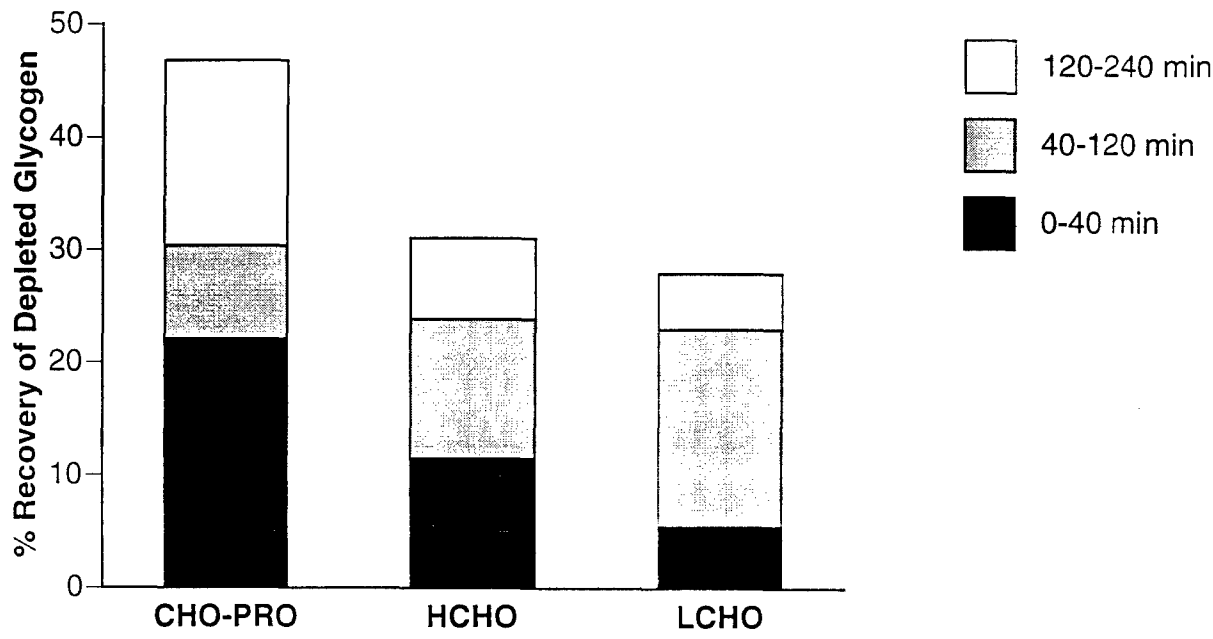
Figure 3. Percent recovery of depleted glycogen stores of the vastus lateralis from 0 to 40, 40 to 120 and 120 to 240 min of recovery. Initial and post exercise muscle glycogen stores were not different among treatments.

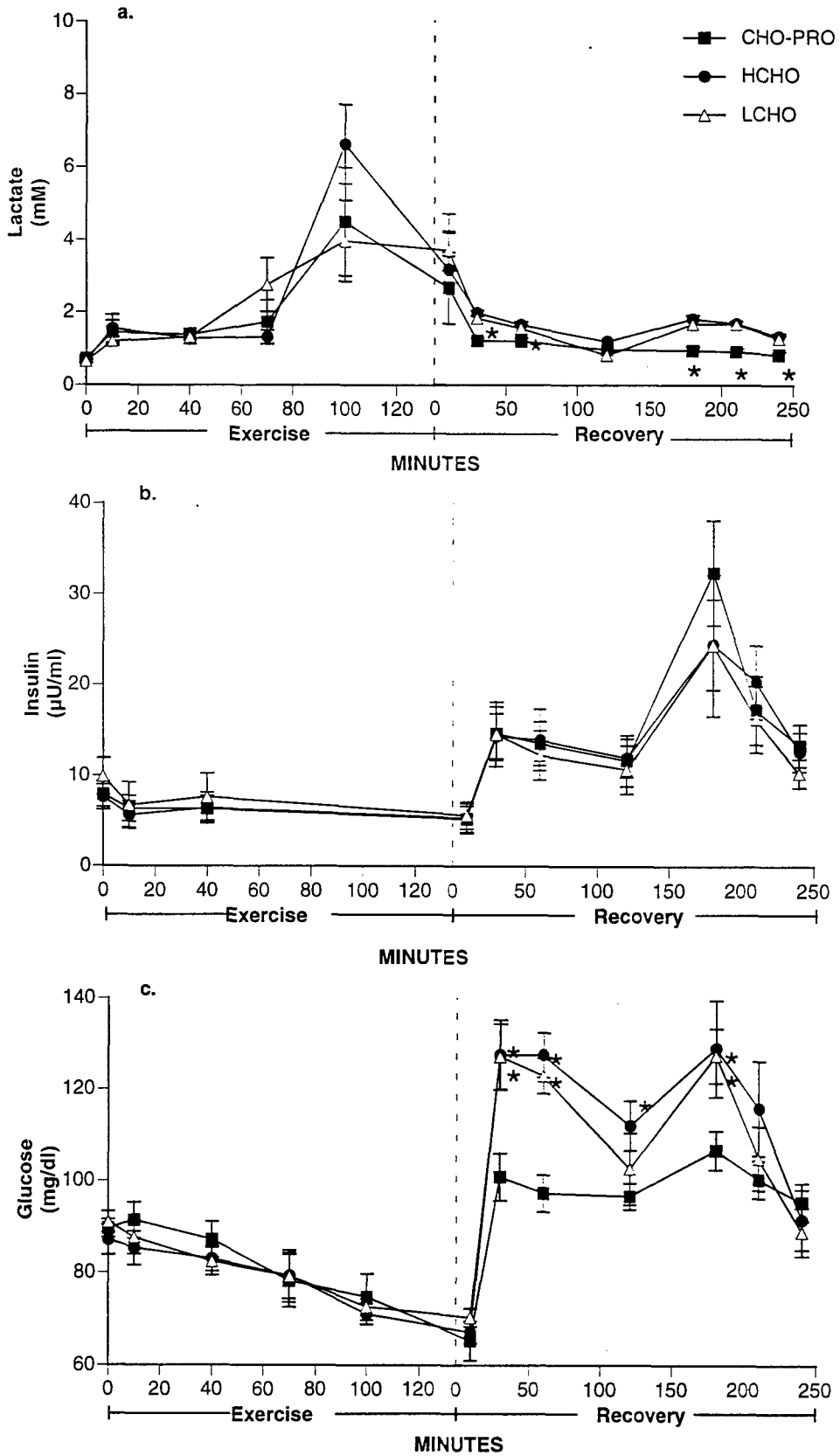
Figure 4. Plasma lactate (**a**), insulin (**b**) and glucose (**c**) during exercise and 4 h of recovery. Treatments were carbohydrate-protein (CHO-PRO), isocarbohydrate (LCHO) and isocaloric carbohydrate (HCHO) supplements provided immediately after and 2 h after exercise. * Significantly different than HCHO and LCHO.

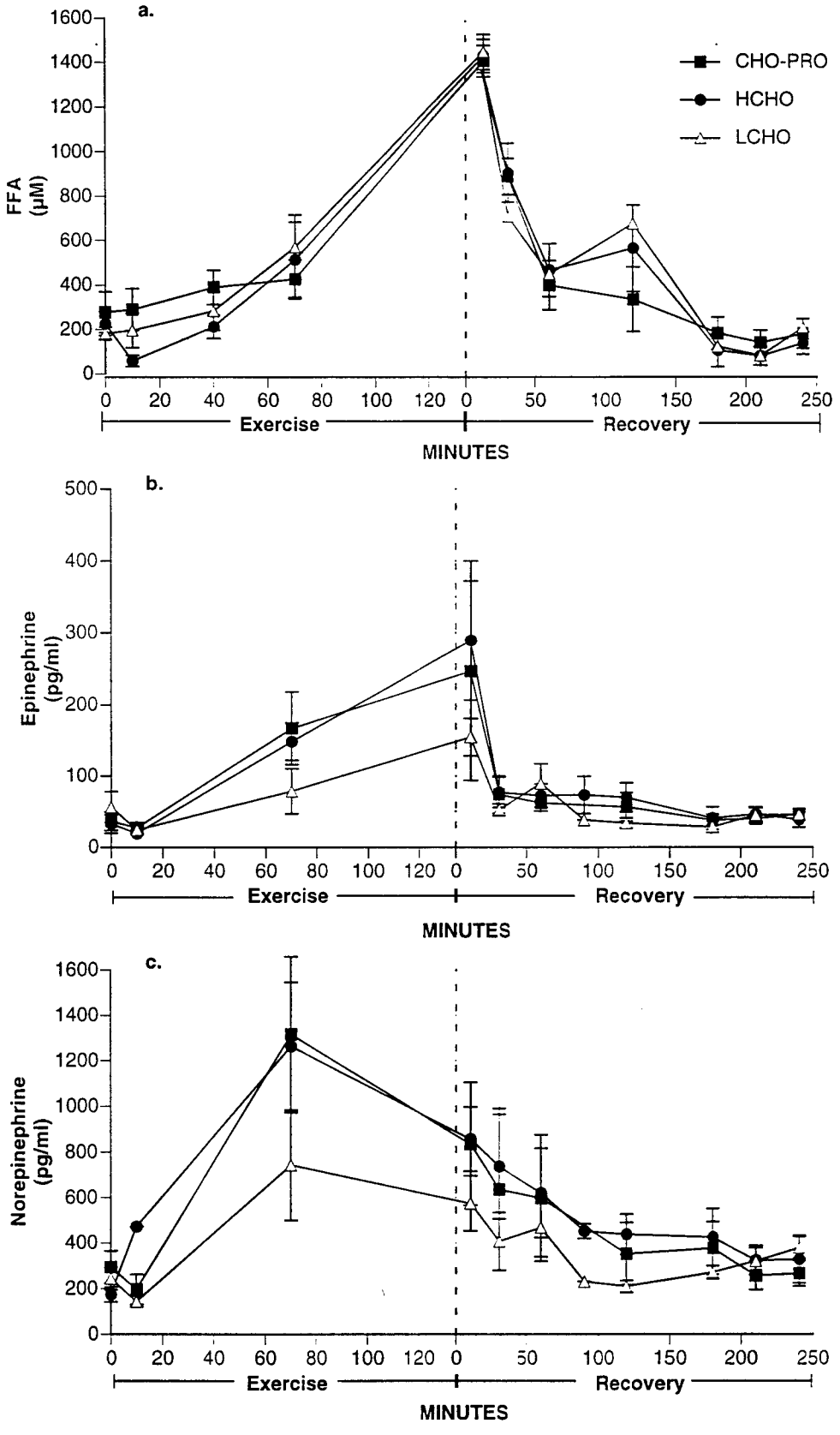
Figure 5. Plasma FFA (**a**), epinephrine (**b**) and norepinephrine (**c**) during exercise and 4 h of recovery. Treatments were carbohydrate-protein (CHO-PRO), isocarbohydrate (LCHO) and isocaloric carbohydrate (HCHO) supplements provided immediately after and 2 h after exercise.


CHO-PRO
HCHO
LCHO
Glycogen Storage (mmol/L)
50
40
30
20
10
0
0
30
60
90
120
150
180
210
240
Post-Exercise Recovery Time
(min)
*
†
*
†
†


Glycogen Storage
mmol/L/4h
50
40
30
20
10
0
*
CHO-PRO
HCHO
LCHO


% Recovery of Depleted Glycogen
50
40
30
20
10
0
CHO-PRO
HCHO
LCHO
120-240 min
40-120 min
0-40 min


a.
CHO-PRO
HCHO
LCHO
Lactate (mM)
10
8
6
4
2
0
0 20 40 60 80 100 120 0 50 100 150 200 250
Exercise
Recovery
MINUTES
b.
Insulin (µU/ml)
40
30
20
10
0
0 20 40 60 80 100 120 0 50 100 150 200 250
Exercise
Recovery
MINUTES
c.
Glucose (mg/dl)
140
120
100
80
60
0 20 40 60 80 100 120 0 50 100 150 200 250
Exercise
Recovery
MINUTES


a.
1600
1400
1200
1000
800
600
400
200
0
FFA (μM)
CHO-PRO
HCHO
LCHO
0 20 40 60 80 100 120 0 50 100 150 200 250
Exercise
Recovery
MINUTES
b.
500
400
300
200
100
0
Epinephrine (pg/ml)
0 20 40 60 80 100 120 0 50 100 150 200 250
Exercise
Recovery
MINUTES
c.
1600
1400
1200
1000
800
600
400
200
0
Norepinephrine (pg/ml)
0 20 40 60 80 100 120 0 50 100 150 200 250
Exercise
Recovery
MINUTES

	MALE T_2 INCREASE [msec]	FEMALE T_2 INCREASE [msec]
15 minutes exercise	3.0±.0.24	4.4±0.25*
60 minutes exercise	3.1±0.16	4.6±0.22*
120 minutes exercise	2.7±0.14	4.3±0.15*
180 minutes exercise	2.7±0.16	5.1±0.18*

Table 1: Total body T_2 increase (upper body + lower body) in male and female populations over the course of 180min of lift & carry exercise. * $p<0.0001$

	MALE T_2 INCREASE [msec]	FEMALE T_2 INCREASE [msec]
15 minutes exercise	3.3±.0.38	4.3±0.34*
60 minutes exercise	3.2±0.29	4.7±0.25*
120 minutes exercise	2.4±0.24	3.9±0.25*
180 minutes exercise	2.8±0.30	4.4±0.35*

Table 2: Upper body T_2 increase in male and female populations over the course of 180min of lift & carry exercise. * $p<0.0001$

	MALE T_2 INCREASE [msec]	FEMALE T_2 INCREASE [msec]
15 minutes exercise	2.9±.0.20	4.4±0.20*
60 minutes exercise	3.010.10	4.5±0.20*
120 minutes exercise	2.8±0.10	4.5±0.10*
180 minutes exercise	2.6±0.10	5.5±0.10*

Table 3: Lower body T_2 increase in male and female populations over the course of 180min of lift & carry exercise. * $p<0.0001$

	15 min EX	60 min EX	120 min EX	180 min EX
Gastrocnemius	----/----	----/----	0.0023 / 0.0158	0.0001 /0.0002
Soleus	----/----	----/----	0.0107 / 0.0006	0.0067 /0.0144
Anterior compartment	----/----	----/----	---- / 0.0006	---- / 0.0012
Vastus lateralis	----/----	0.0132 / 0.0170	---- / 0.0156	0.0233 / 0.0071
Rectus femoris	0.0331/----	0.0300 / 0.0183	0.0319 / 0.0251	0.0117 / 0.0093
Vastus intermedius	----/----	0.0078 / ----	----/----	0.0454 / 0.0308
Vastus medalis	----/----	----/----	---- / 0.0194	0.0304 / 0.0028
Biceps femoris	----/----	0.0136 / 0.0130	0.0035 / 0.0078	0.0004 / 0.0002
Semimembranosus	----/----	0.0318 / ----	0.0007 / 0.0009	0.0004 / 0.0008
Semitendinosus	----/----	0.0065 / ----	---- / 0.0480	0.0072 / 0.0007
Sartorius	----/----	0.0007 / 0.0119	----/----	0.0389 / 0.0017
Gluteus maximus	----/----	0.0358 / 0.0192	0.0065 / 0.0387	0.0035 / 0.0052
Gluteus medius	----/----	----/----	----/----	0.0187 / 0.0132
Gluteus minimus	----/----	----/----	----/----	0.0159 / ----

Table 4 Lower body: Degree of significance of the difference between T_2 increases in the male versus female populations, over the course of 180min of exercise. Muscles are paired (male muscle x versus female muscle x). Contralateral muscles are presented as ----/---- = left / right

	15 min EX	60 min EX	120 min EX	180 min EX
Deltoids	----/----	----/----	0.0357 / ----	---- / 0.0149
Triceps	----/----	----/----	----/----	----/----
Biceps brachialis	0.0073 / 0.0079	0.0090 / 0.0030	0.0270 / 0.0264	0.0078 / 0.0037
Superior forearm	0.0298 / ----	----/----	----/----	---- / 0.0001
Inferior forearm	----/----	----/----	----/----	----/----
Trapezius	----/----	----/----	----/----	----/----
Pectoralis major	----/----	----/----	----/----	----/----
Pectoralis minor	----/----	----/----	----/----	----/----
Latissimus dorsi	----/----	----/----	----/----	----/----
Rectus abdominus	----/----	----/----	----/----	----/----
Lower back	----/----	----/----	----/----	----/----

Table 5 Upper body: Degree of significance of the difference between T_2 increases in the male versus female populations, over the course of 180min of exercise. Muscles are paired (male muscle x versus female muscle x). Contralateral muscles are presented as ----/---- = left / right

Figure 1

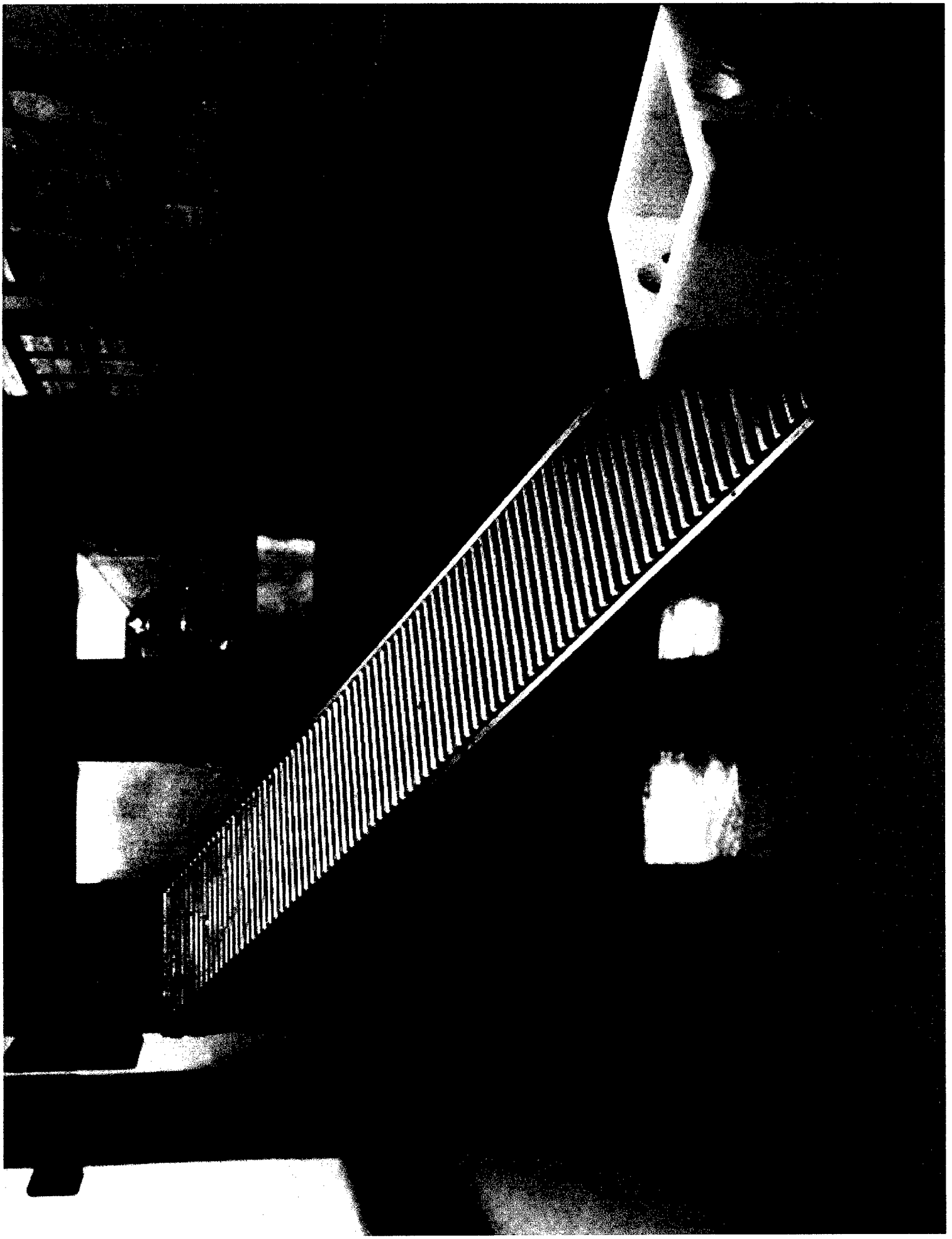
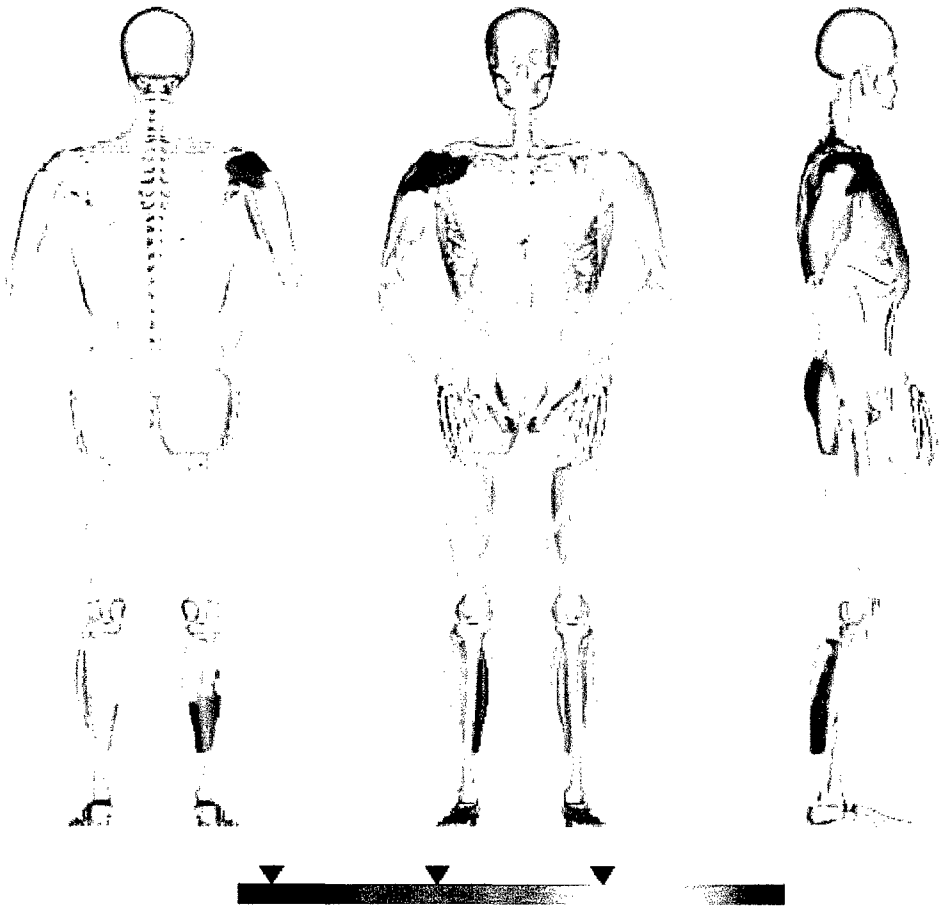


Figure 2a



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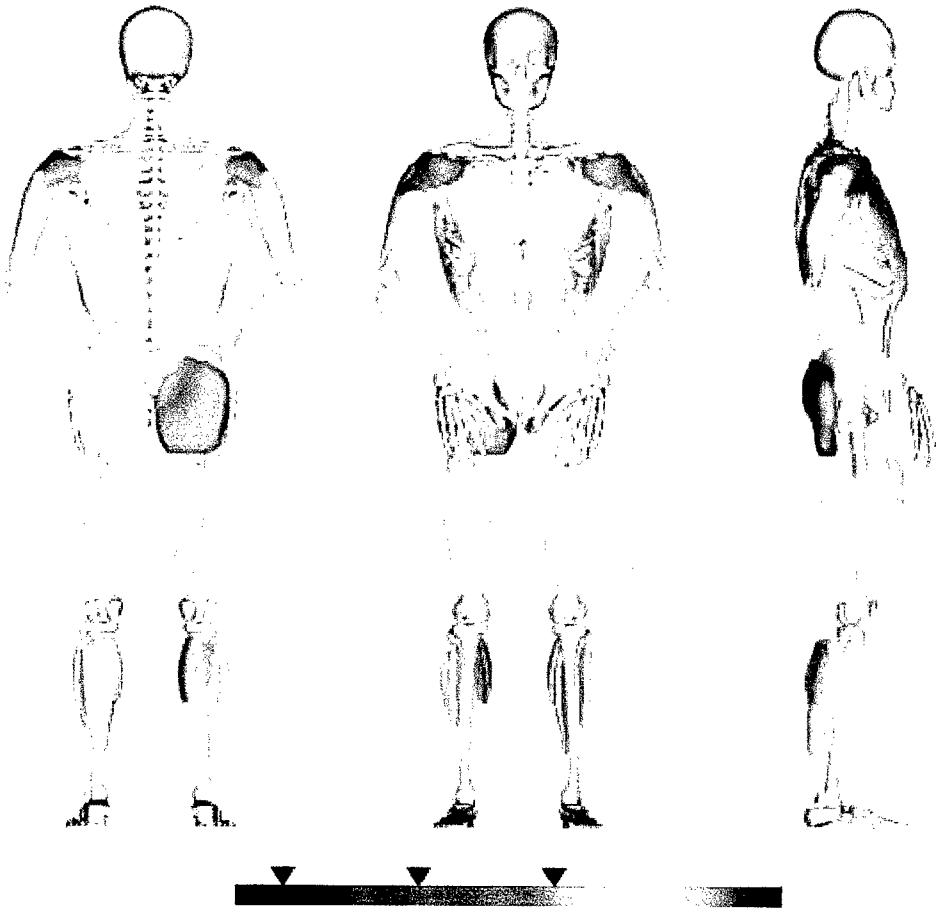

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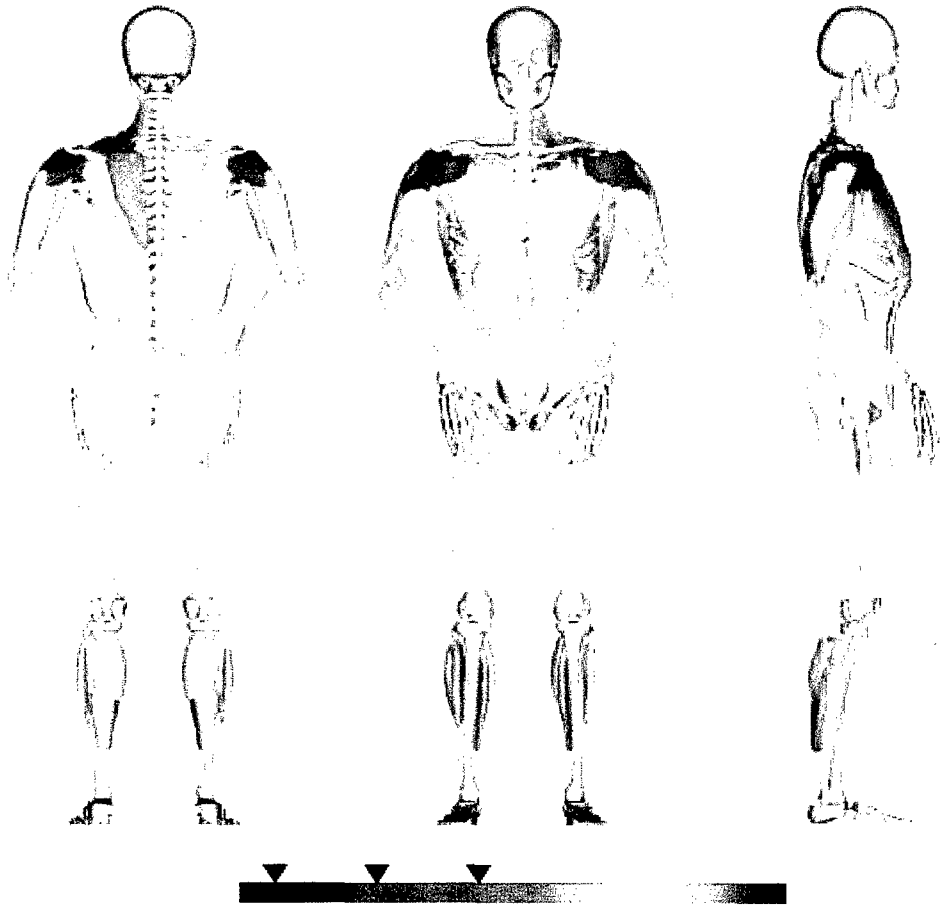

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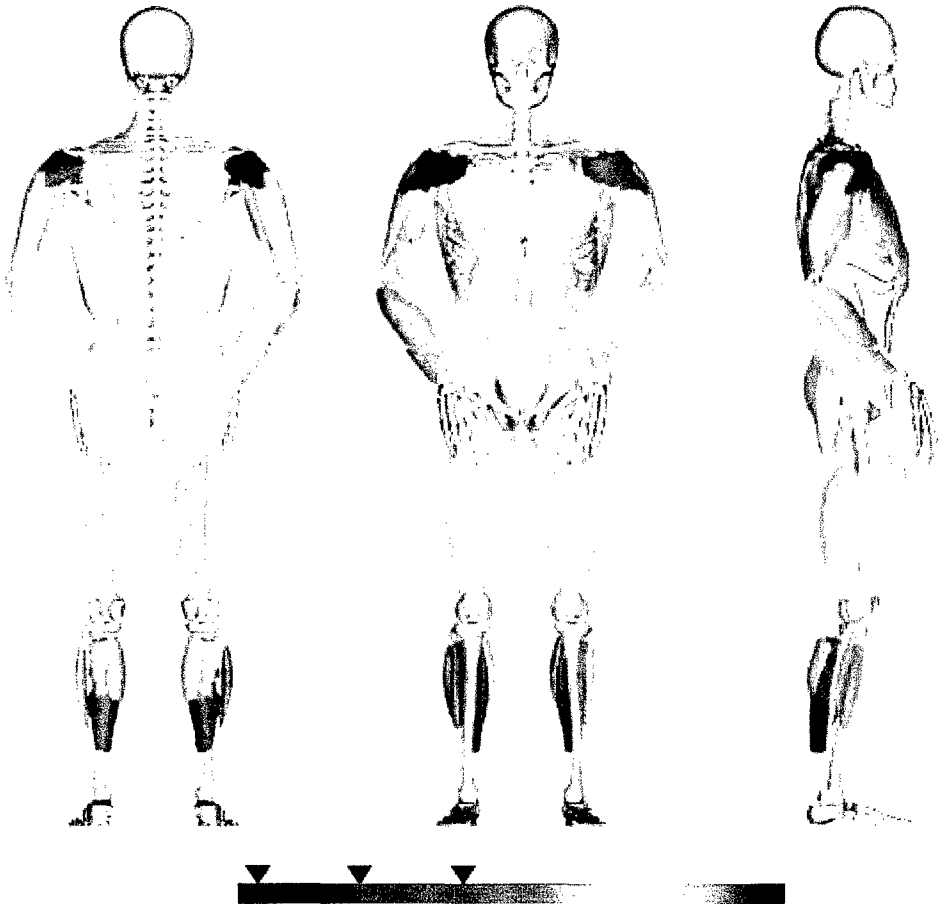
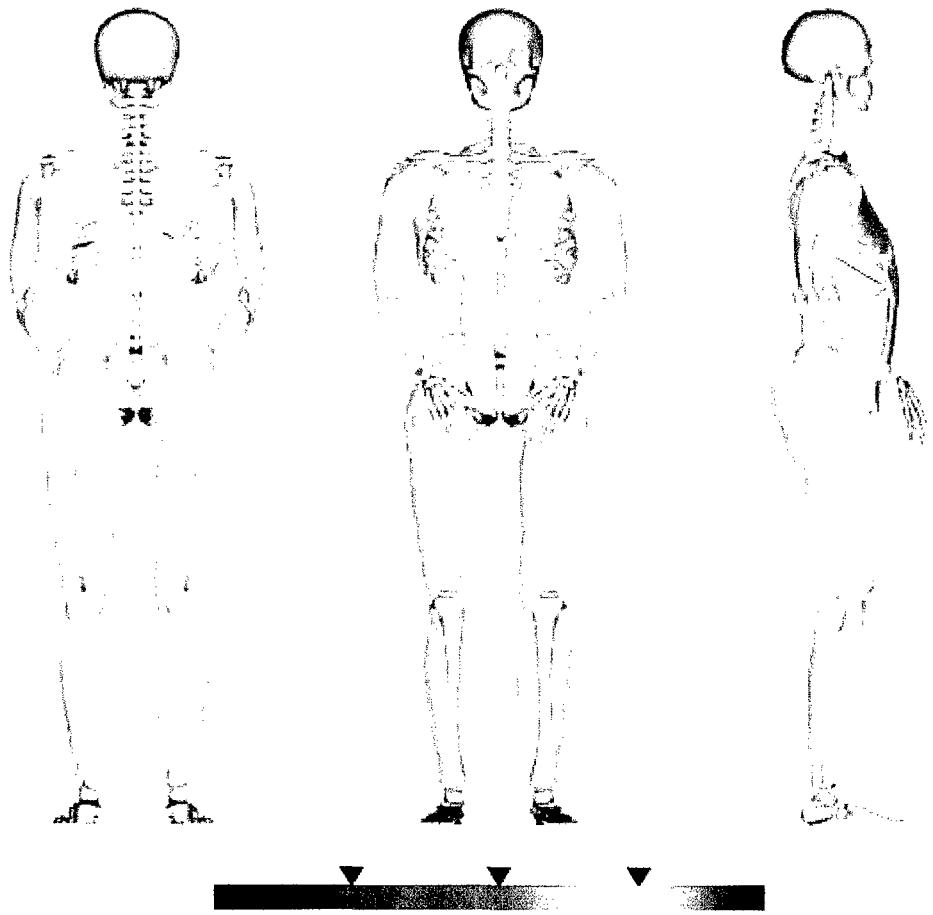
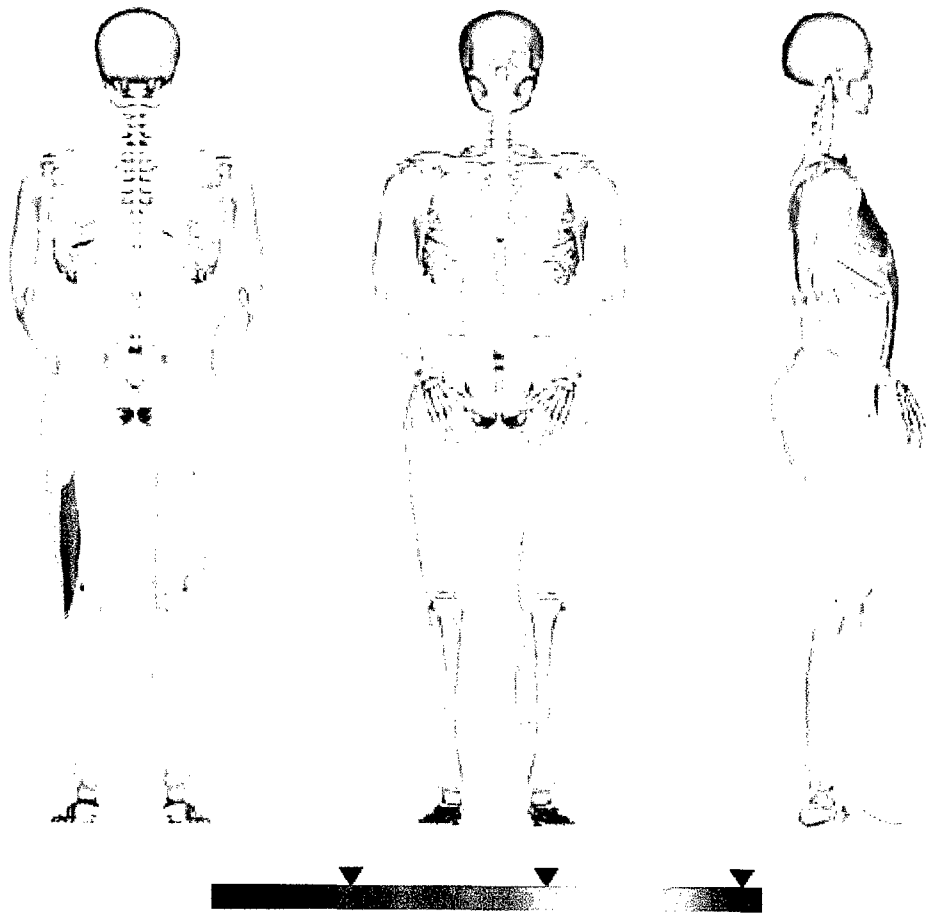


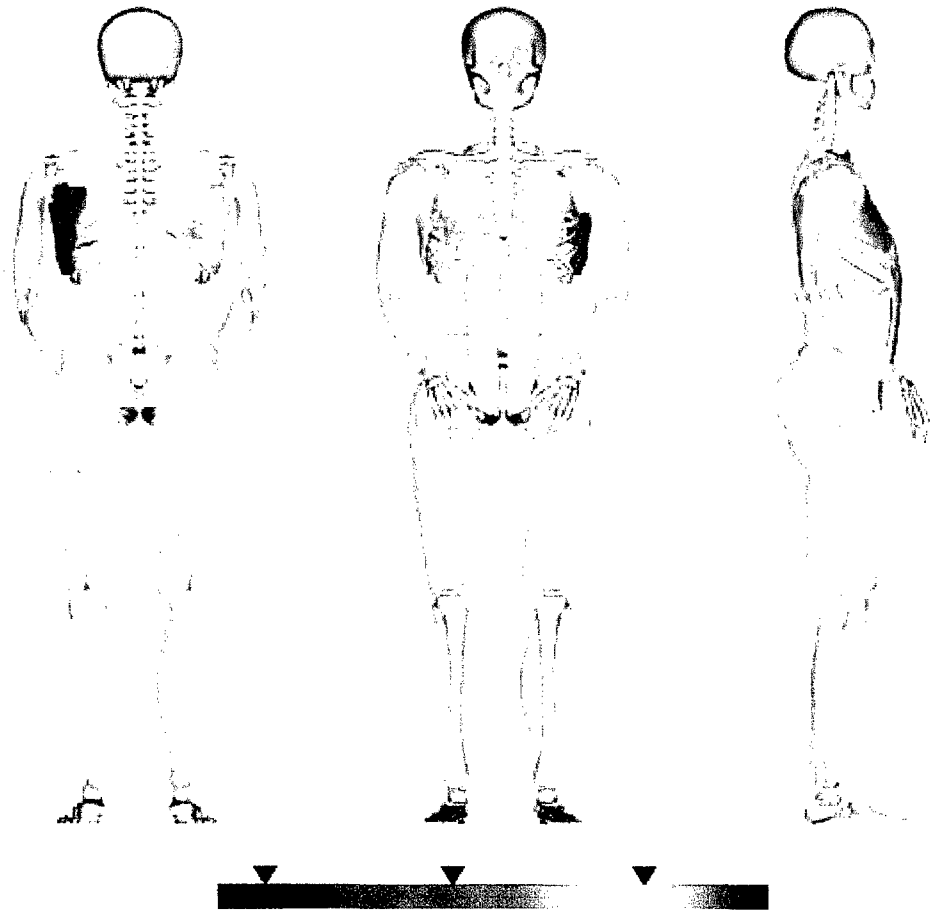
Figure 3a



3b



3c



3d

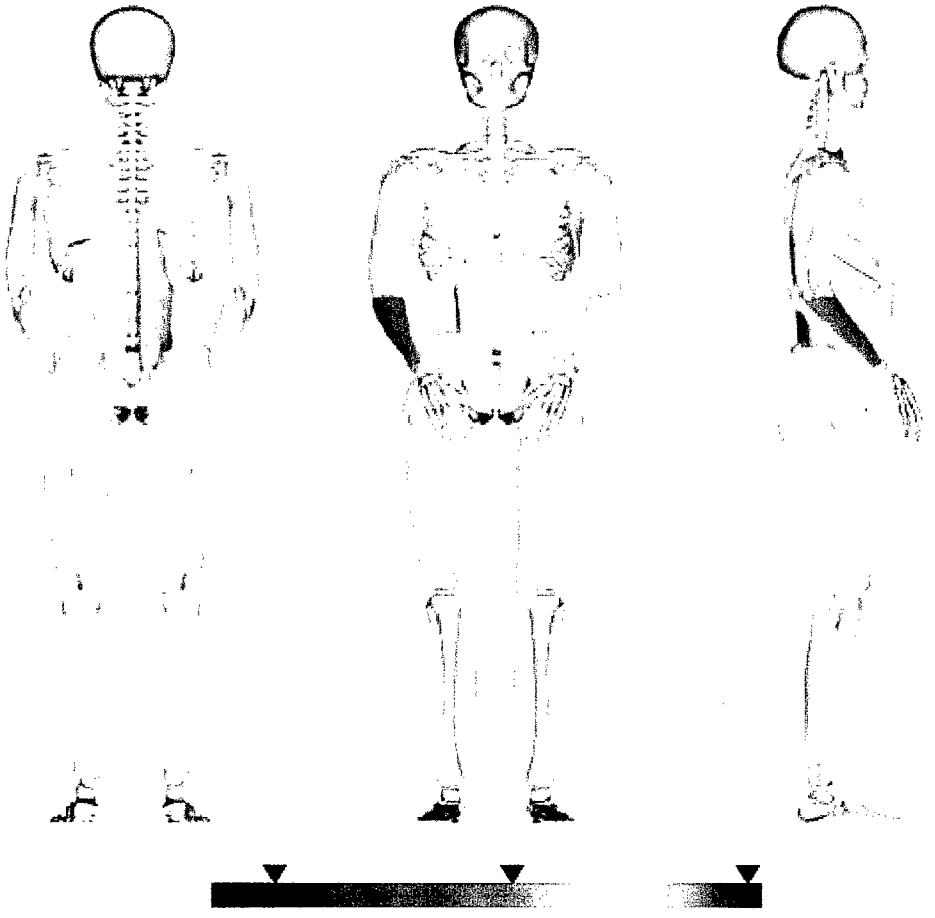


Figure 4:

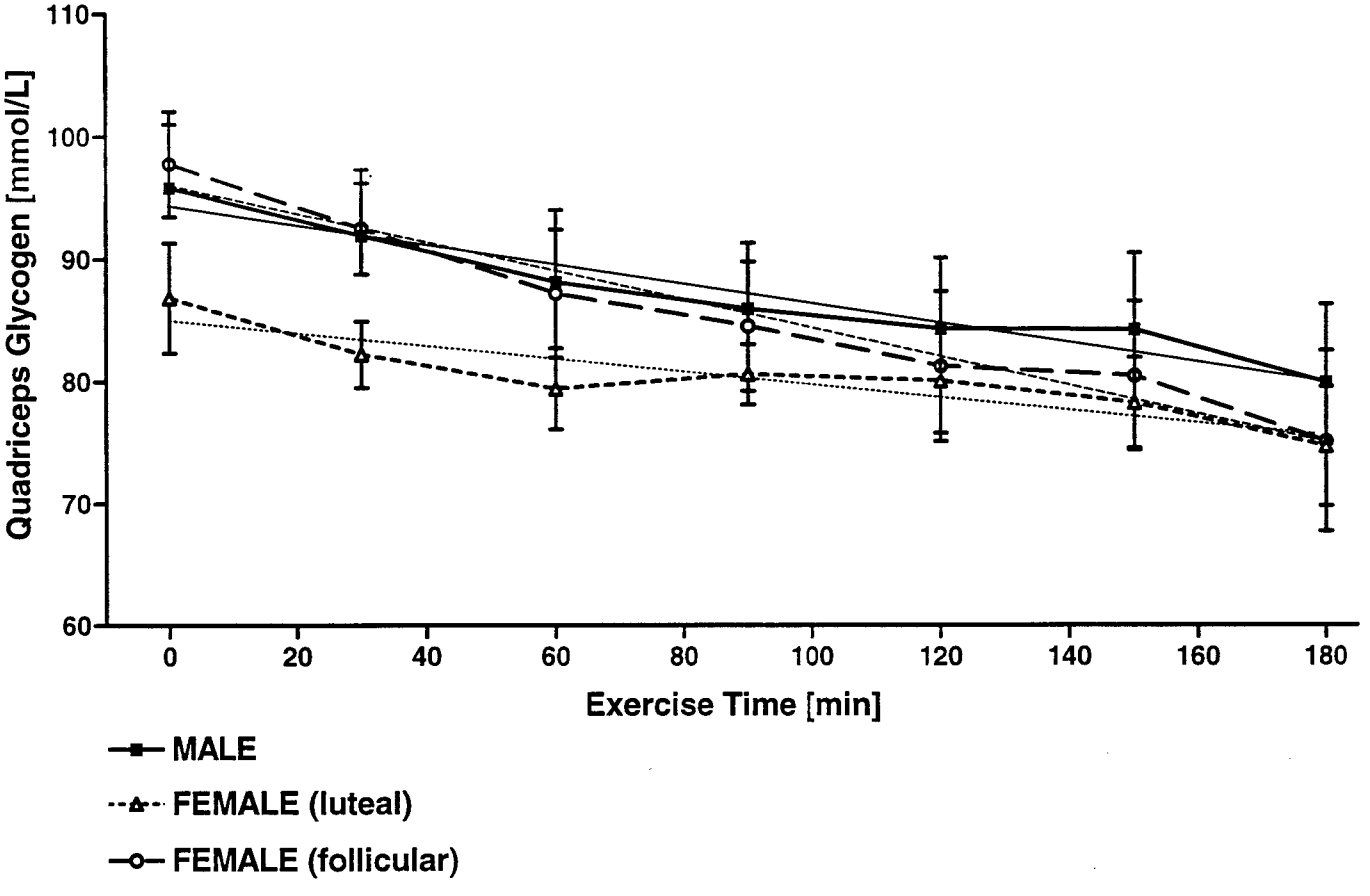
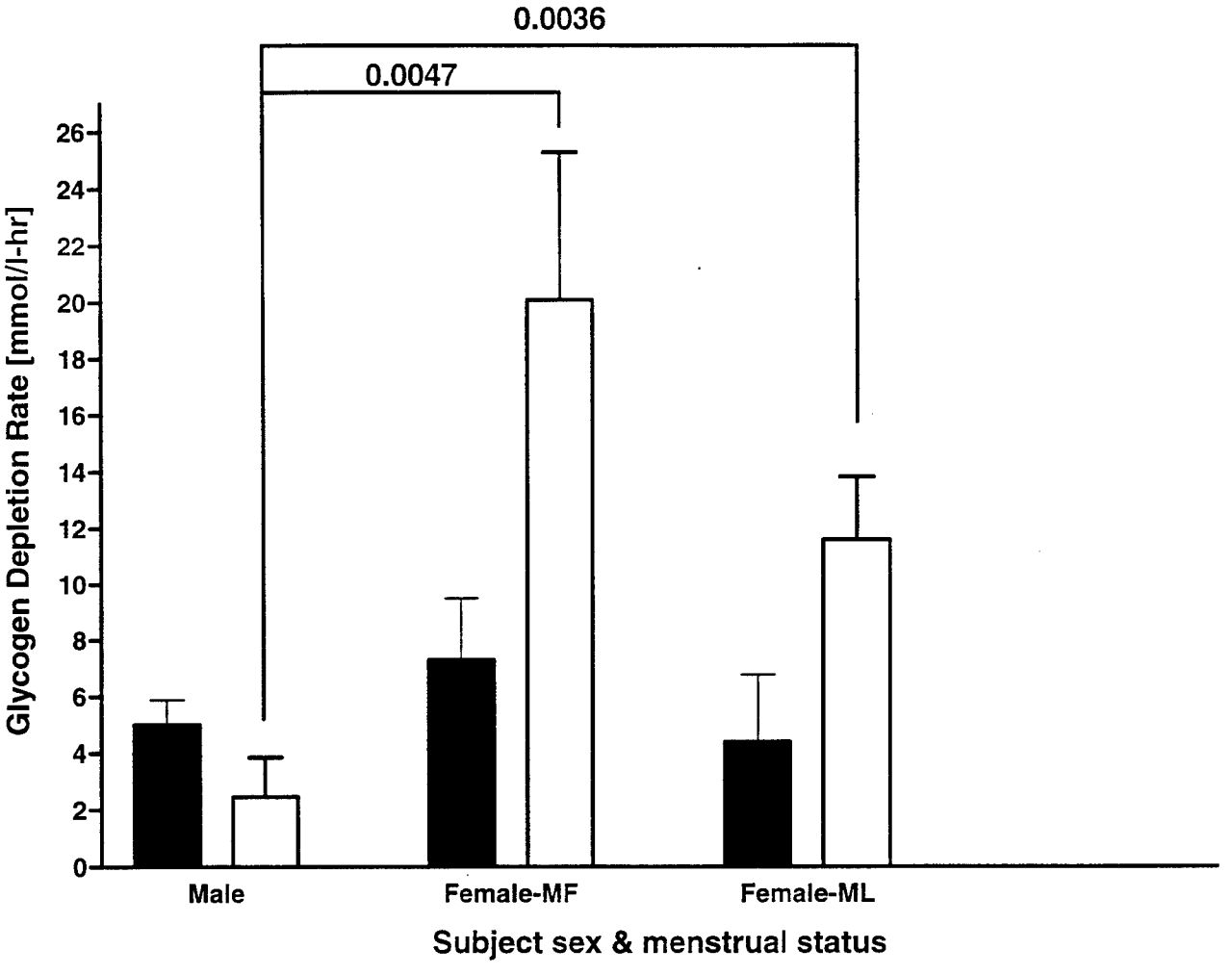

Quadriceps Glycogen [mmol/L]
110
100
90
80
70
60
0
20
40
60
80
100
120
140
160
180
Exercise Time [min]
MALE
FEMALE (luteal)
FEMALE (follicular)

Figure 5:



*p<0.04 Quads vs Biceps

Figure 6:

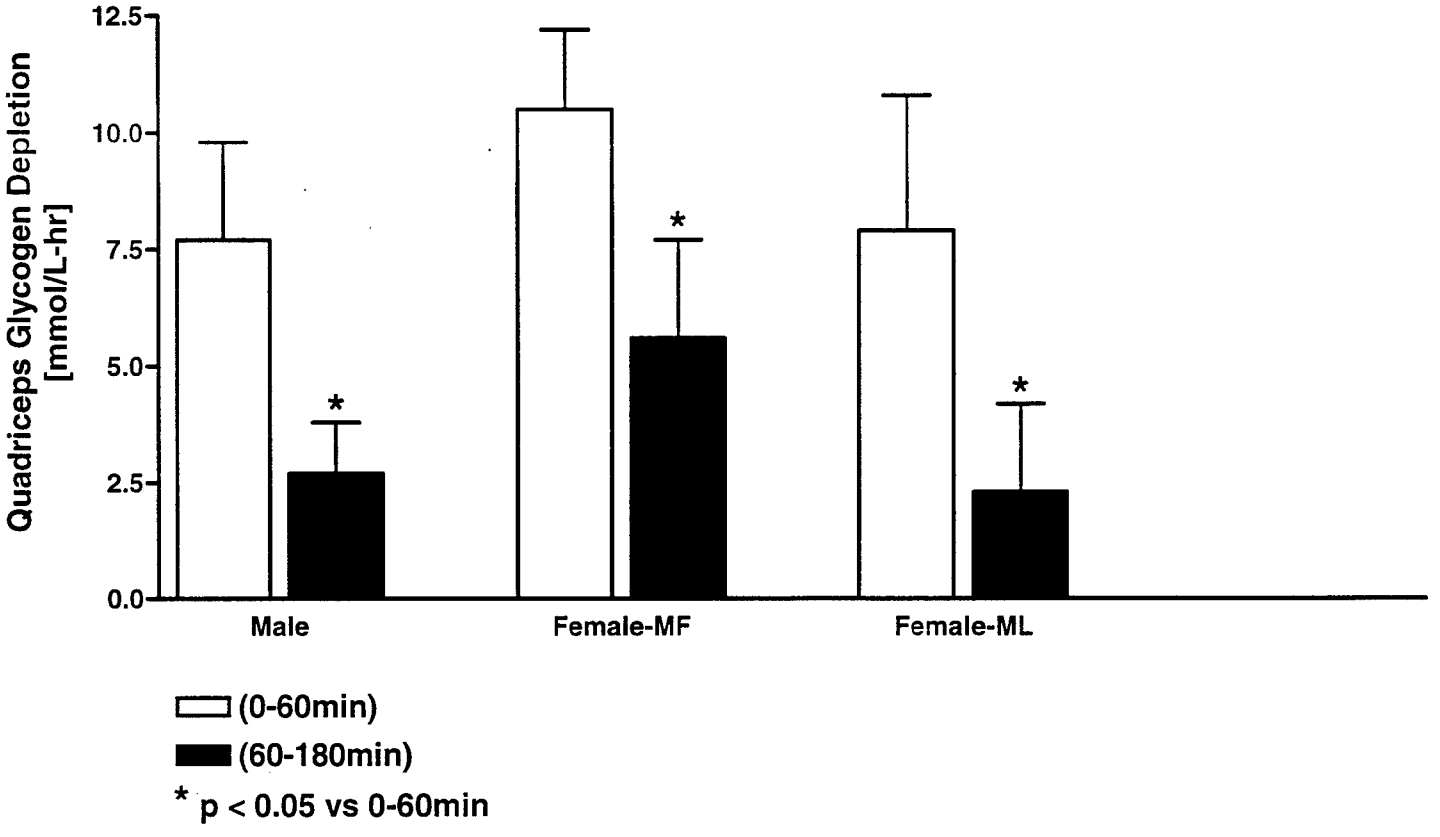

Quadriceps Glycogen Depletion
[mmol/L-hr]
12.5
10.0
7.5
5.0
2.5
0.0
*
*
*
Male
Female-MF
Female-ML
(0-60min)
(60-180min)
* p < 0.05 vs 0-60min

Figure 7:

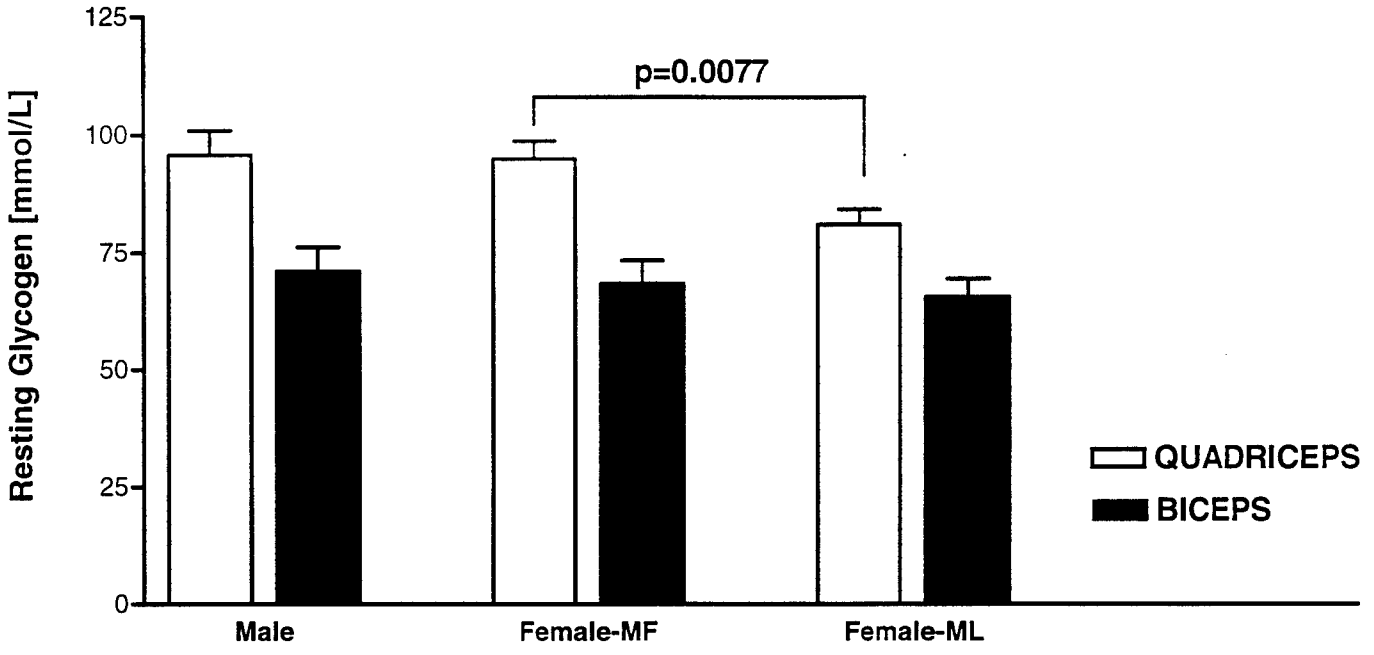

Resting Glycogen [mmol/L]
125
100
75
50
25
0
p=0.0077
QUADRICEPS
BICEPS
Male
Female-MF
Female-ML

Figure 8:

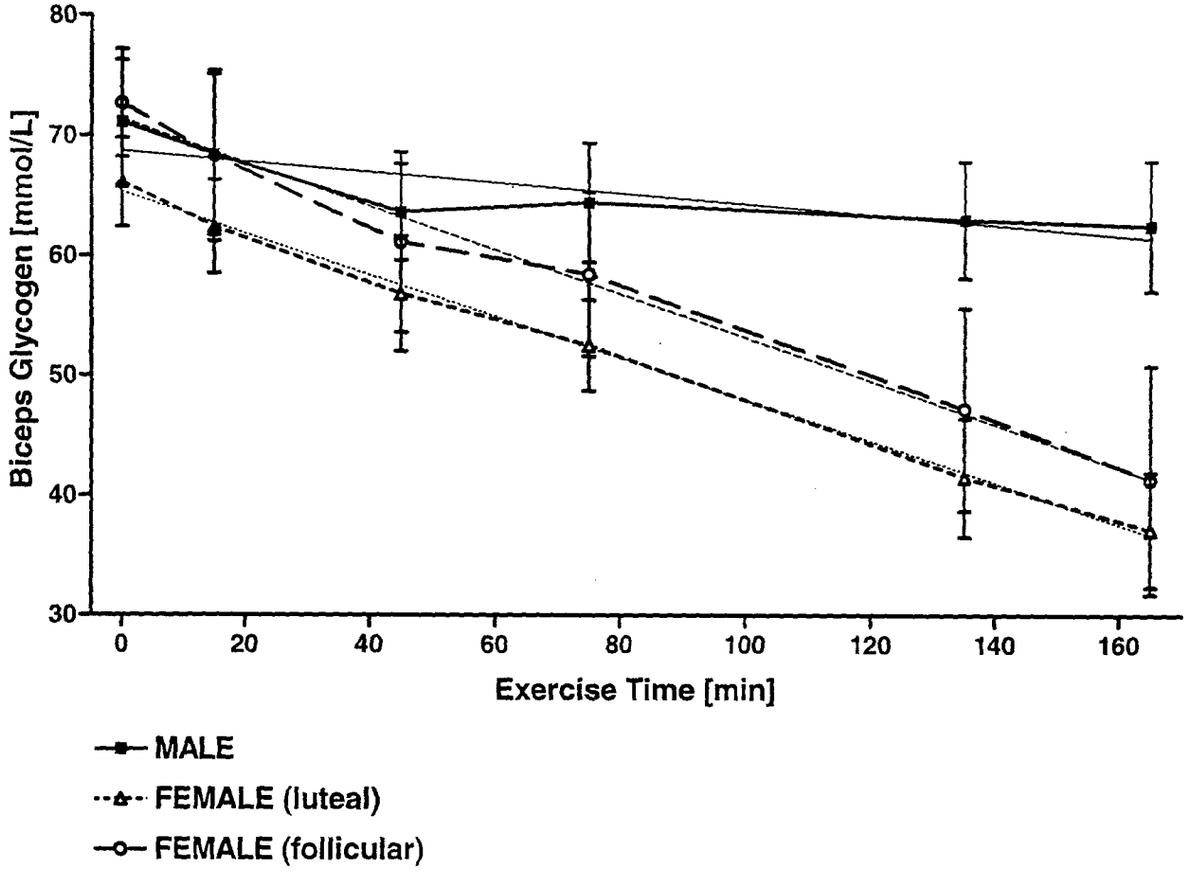

Biceps Glycogen [mmol/L]
80
70
60
50
40
30
0
20
40
60
80
100
120
140
160
Exercise Time [min]
MALE
FEMALE (luteal)
FEMALE (follicular)

Figure 9:

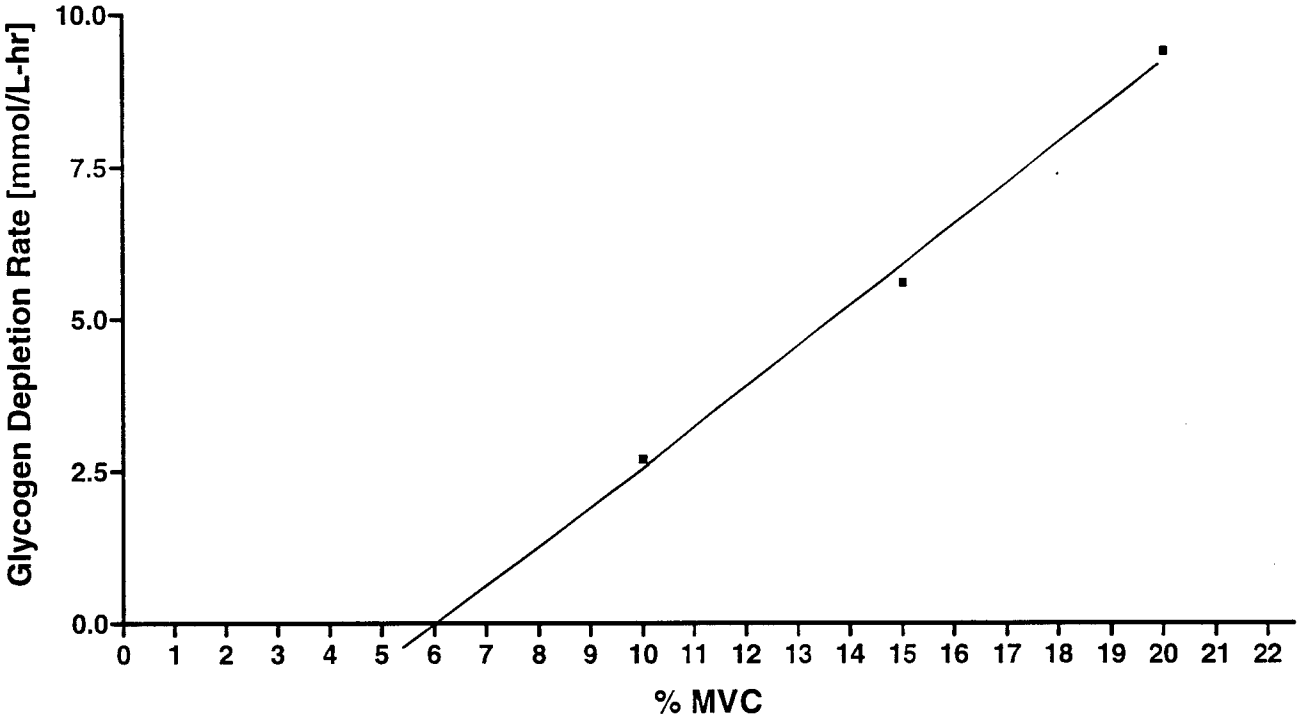

Glycogen Depletion Rate [mmol/L-hr]
10.0
7.5
5.0
2.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
% MVC

Figure 10:

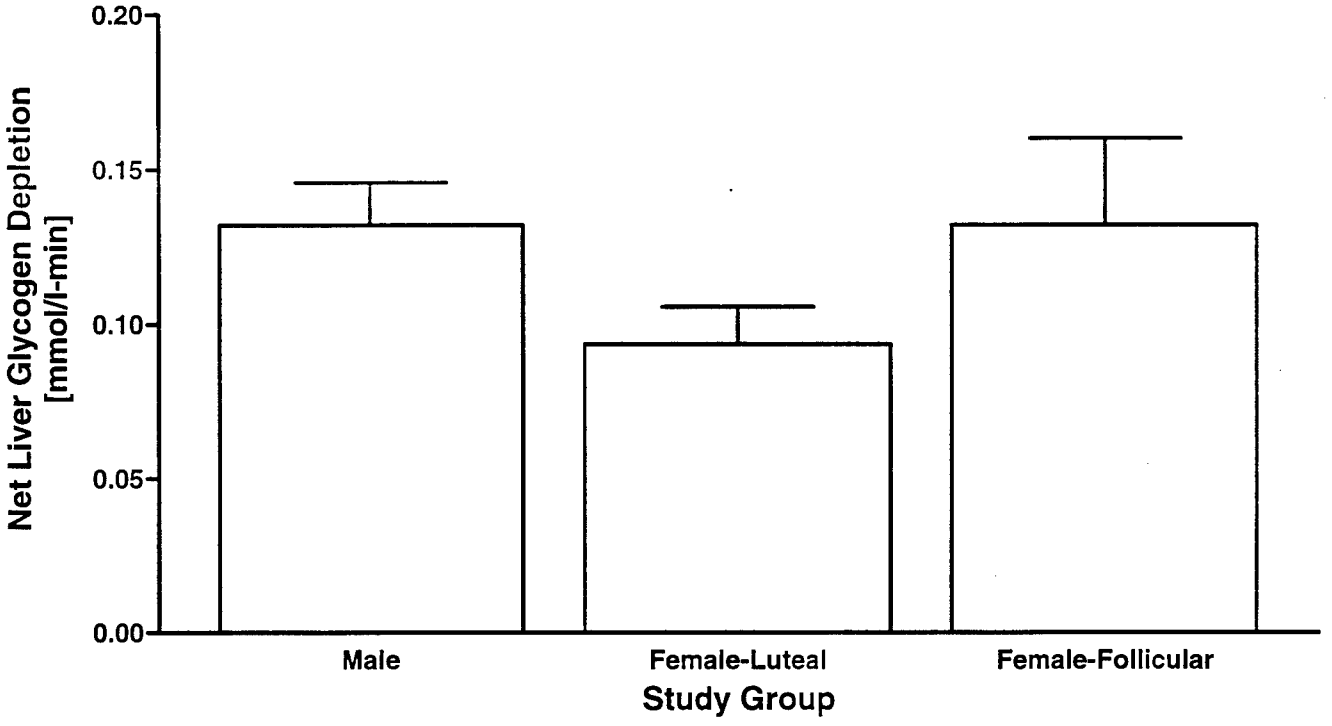
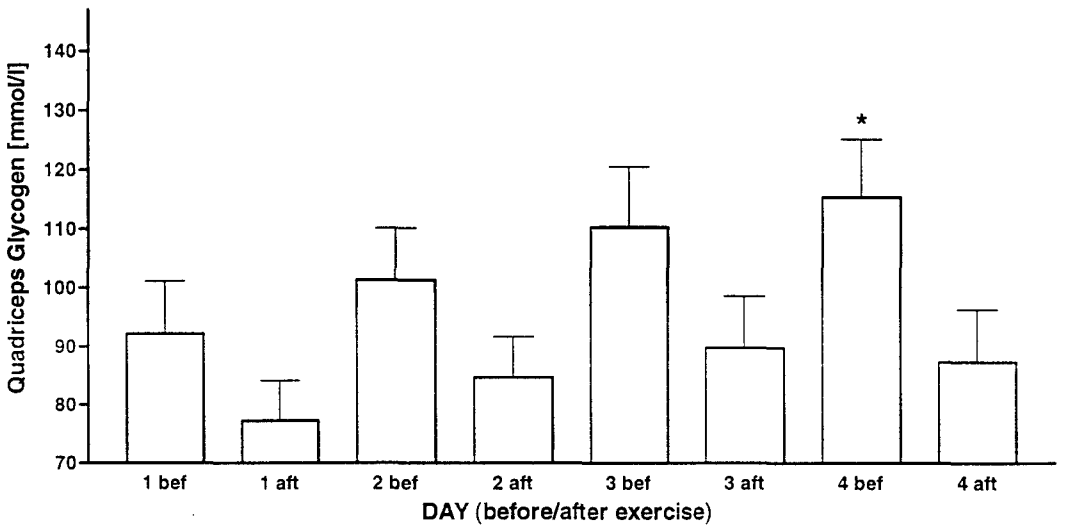

0.20
0.15
0.10
0.05
0.00
Net Liver Glycogen Depletion
[mmol/l-min]
Male
Female-Luteal
Female-Follicular
Study Group

Figure 11:

* paired P<0.05 vs day 1 pre-exercise



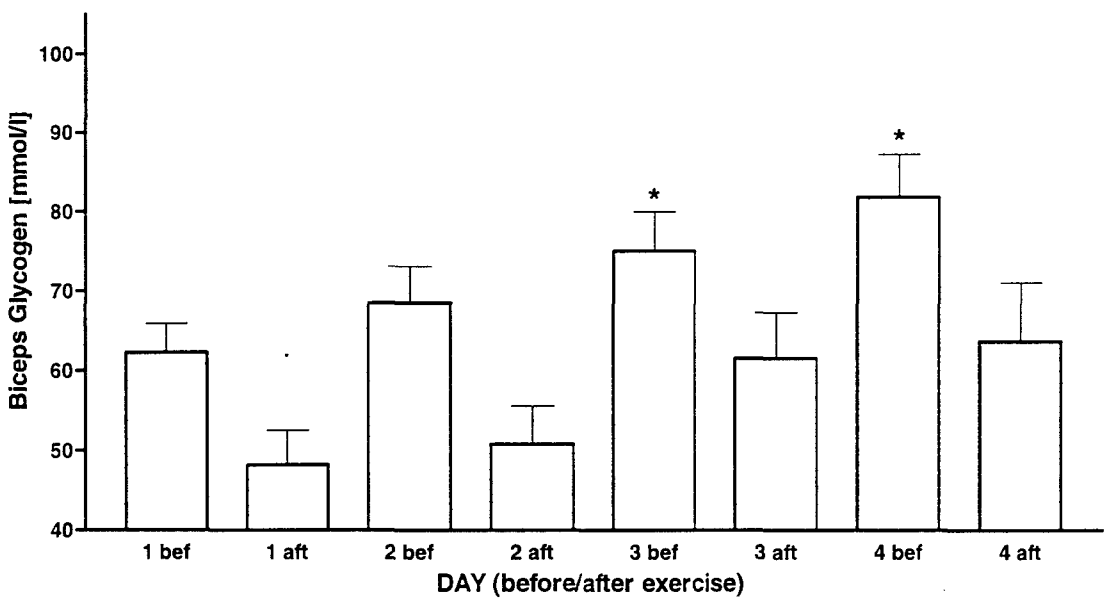
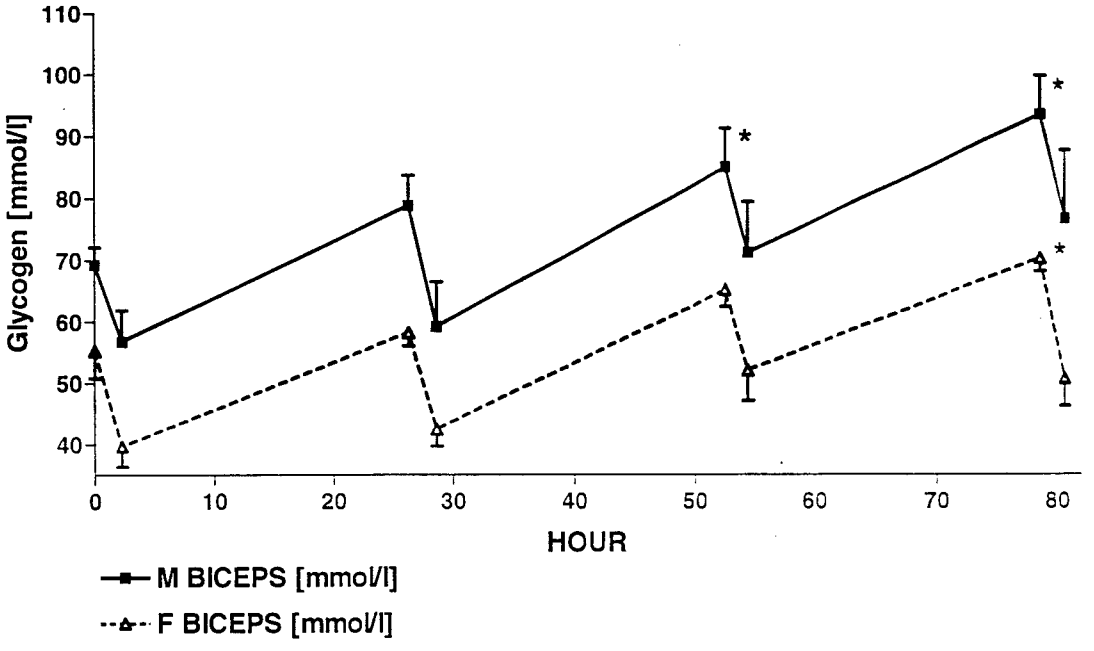
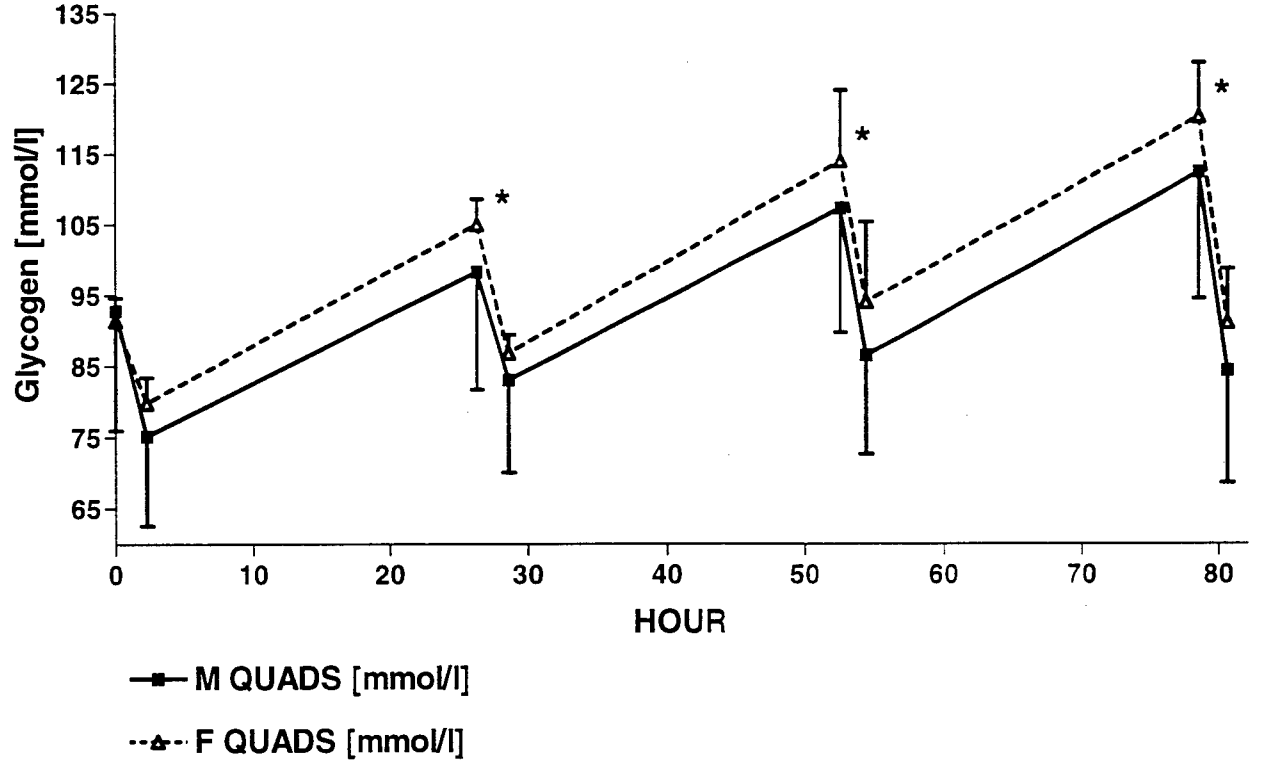


Figure 12:

* paired p<0.05 vs day 1





DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 28 July 03

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
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